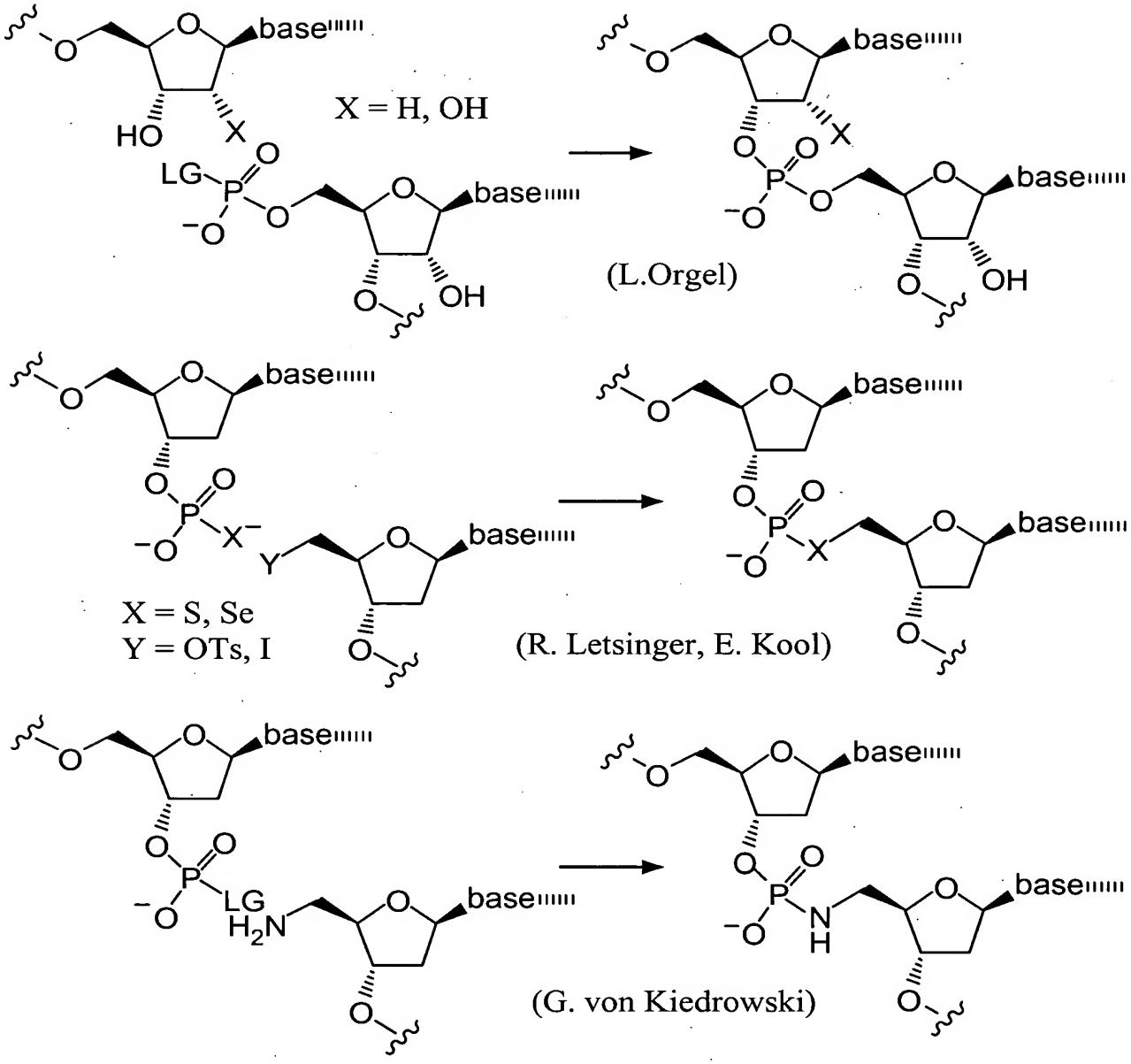


FIG. 1A

FIG. 1A	FIG. 1B

FIG. 1

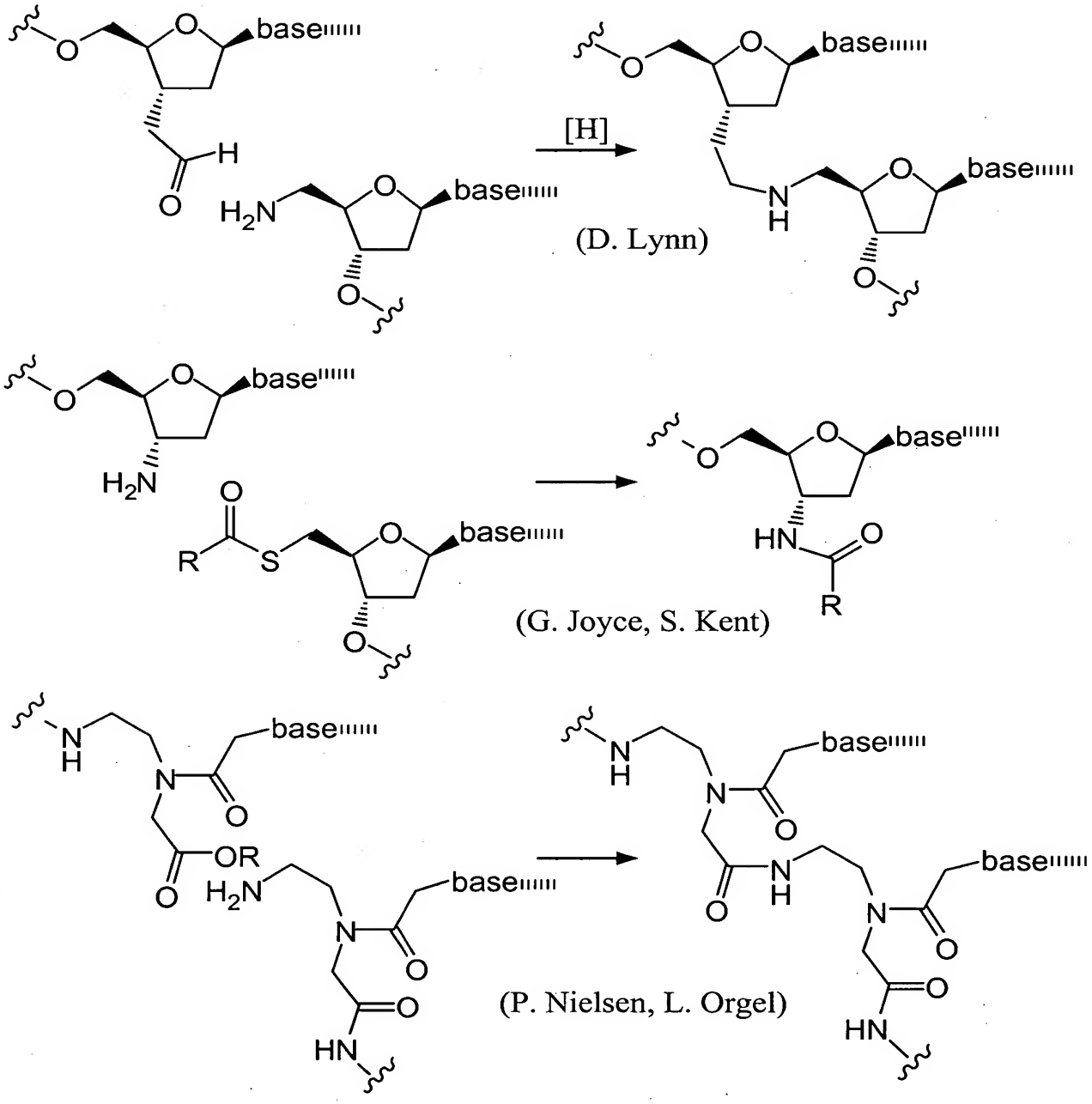


FIG. 1B

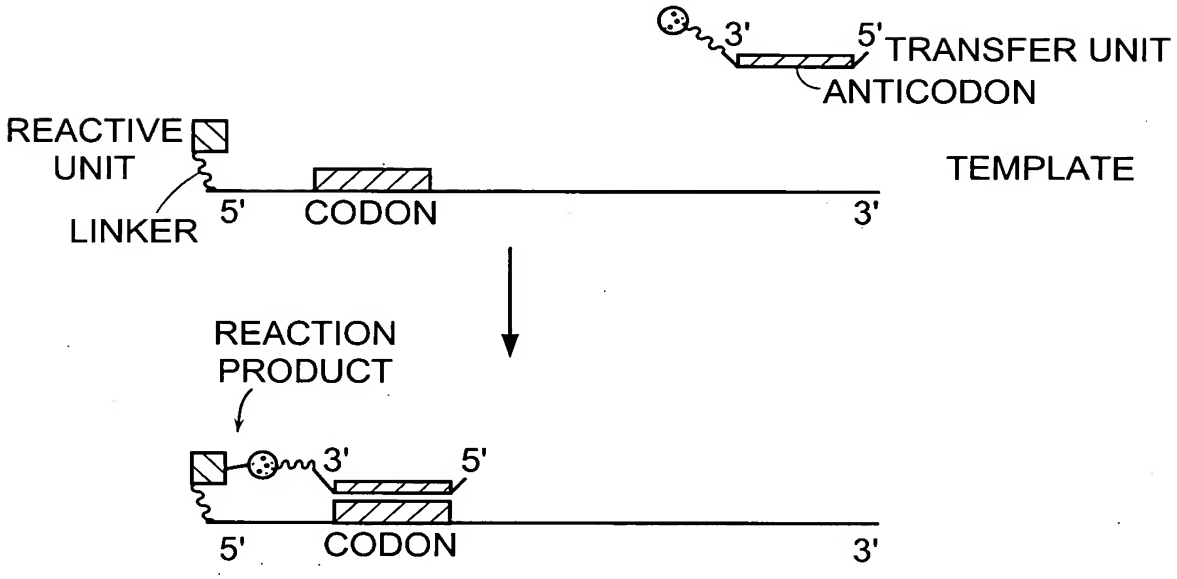

3'
5'
TRANSFER UNIT
ANTICODON
REACTIVE UNIT
LINKER
5'
CODON
3'
TEMPLATE
REACTION PRODUCT
3'
5'
5'
CODON
3'

FIG. 2

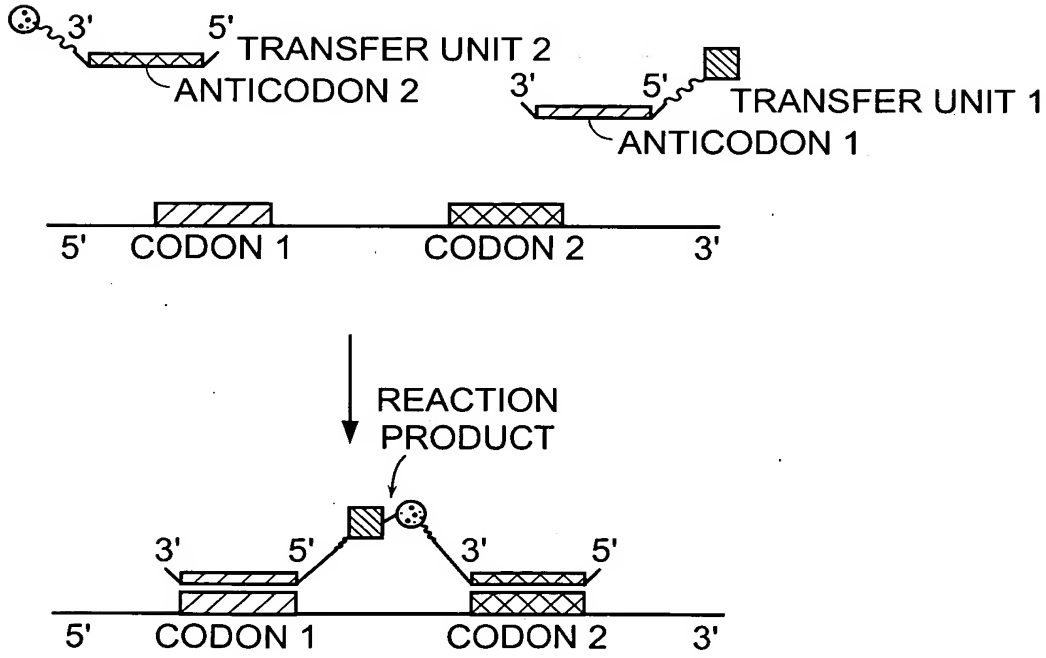

3'
5'
TRANSFER UNIT 2
ANTICODON 2
3'
5'
TRANSFER UNIT 1
ANTICODON 1
5'
CODON 1
CODON 2
3'
REACTION PRODUCT
3'
5'
3'
5'
5'
CODON 1
CODON 2
3'

FIG. 3

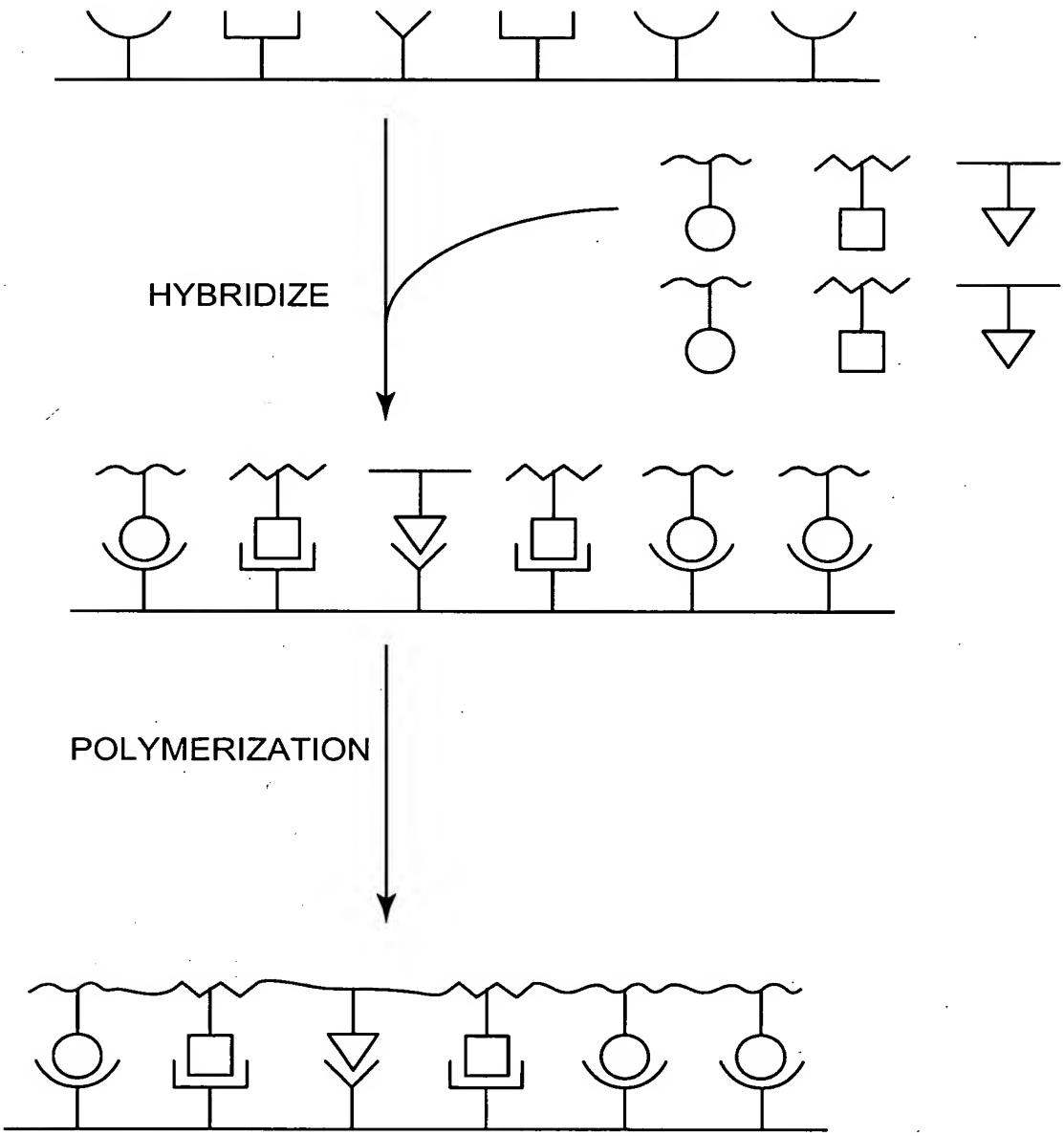


FIG. 4

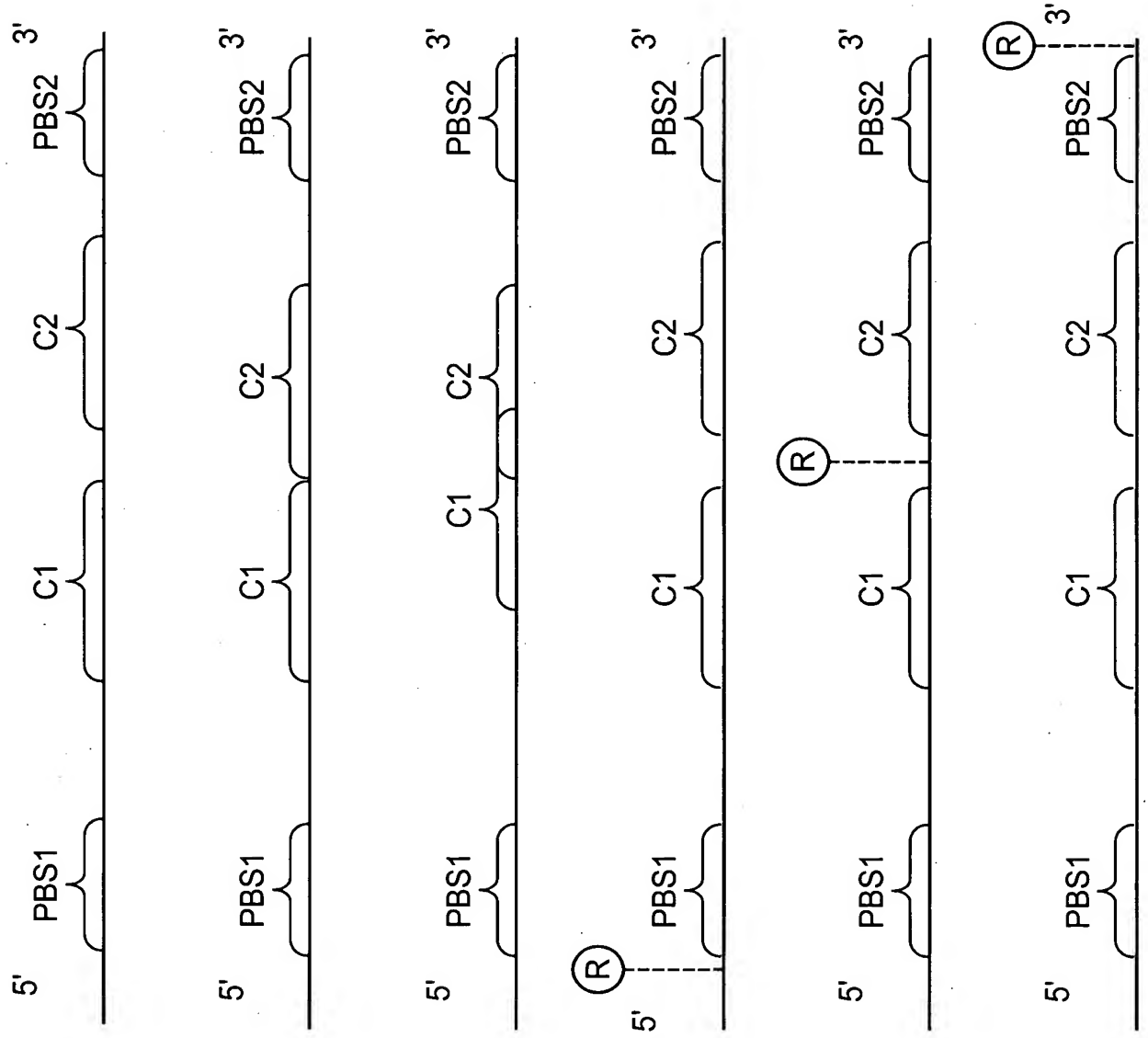


FIG. 5A

FIG. 5B

FIG. 5C

FIG. 5D

FIG. 5E

FIG. 5F

FIG. 6A

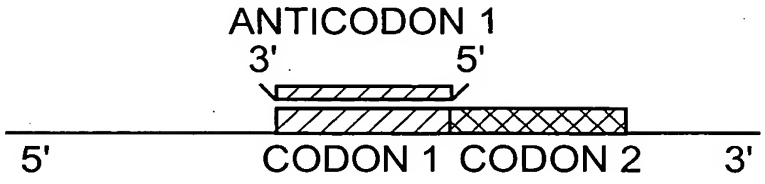


FIG. 6B

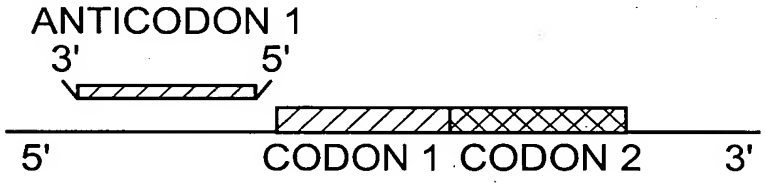


FIG. 6C

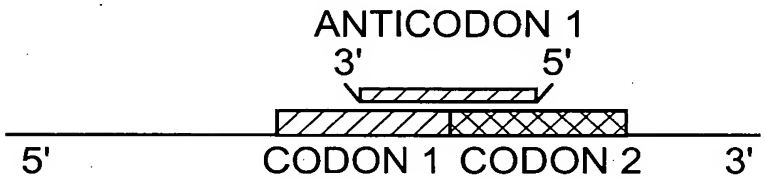


FIG. 6D

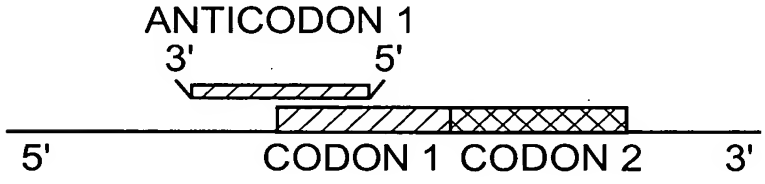
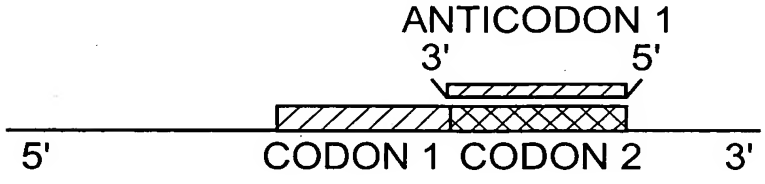


FIG. 6E



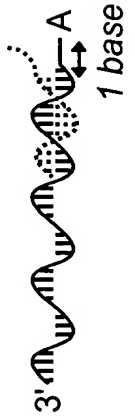


FIG. 7A

FIG. 7B

FIG. 7C

FIG. 7D

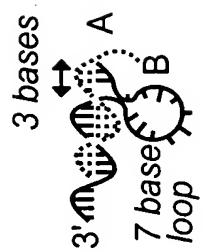


FIG. 7E

FIG. 7F

FIG. 7G

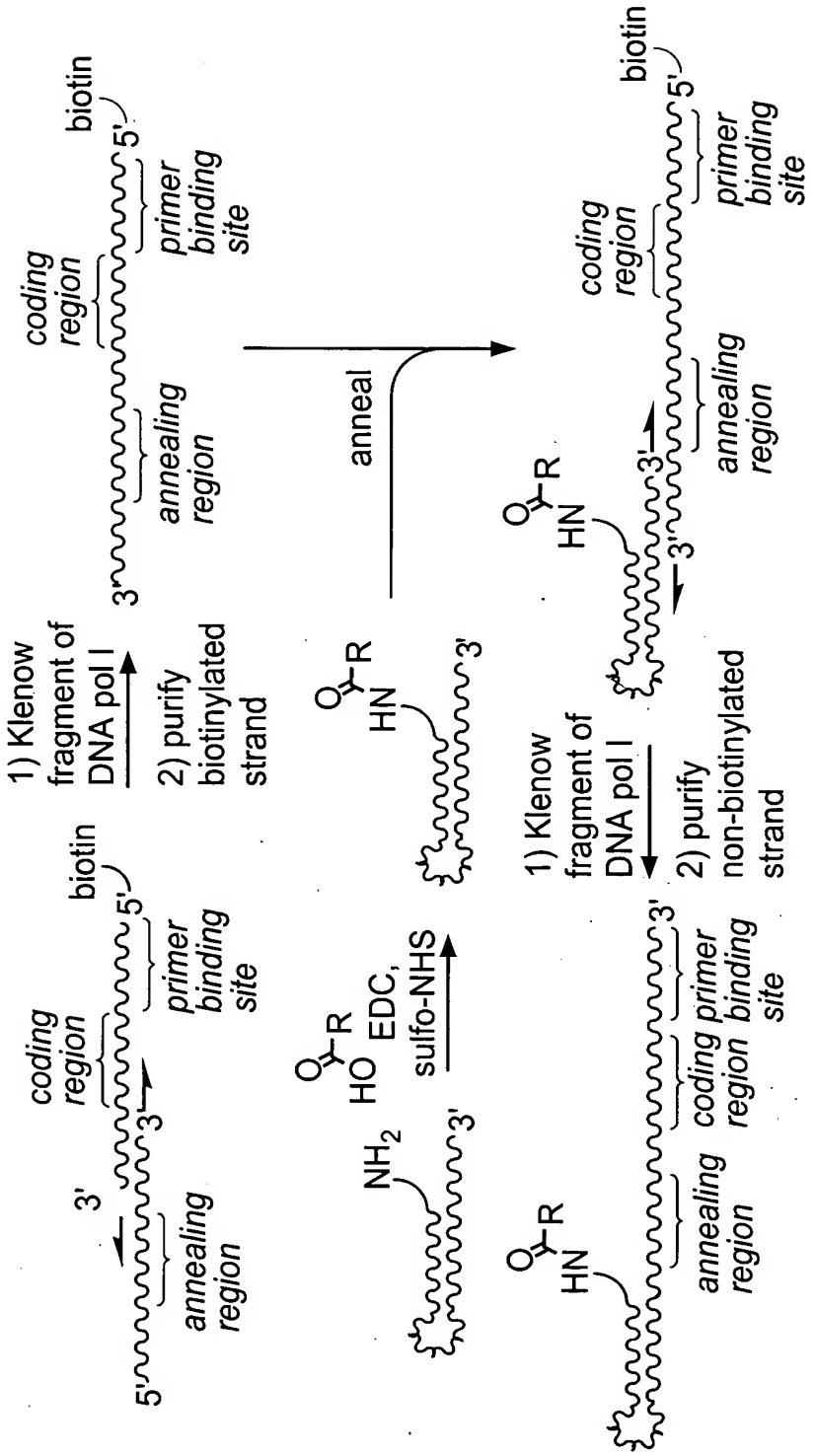

biotin
5'
primer binding site
coding region
3'
annealing region
1) Klenow fragment of DNA pol I
2) purify biotinylated strand
anneal
EDC, sulfo-NHS
1) Klenow fragment of DNA pol I
2) purify non-biotinylated strand
HO
NH2
HN
R
O

FIG. 8

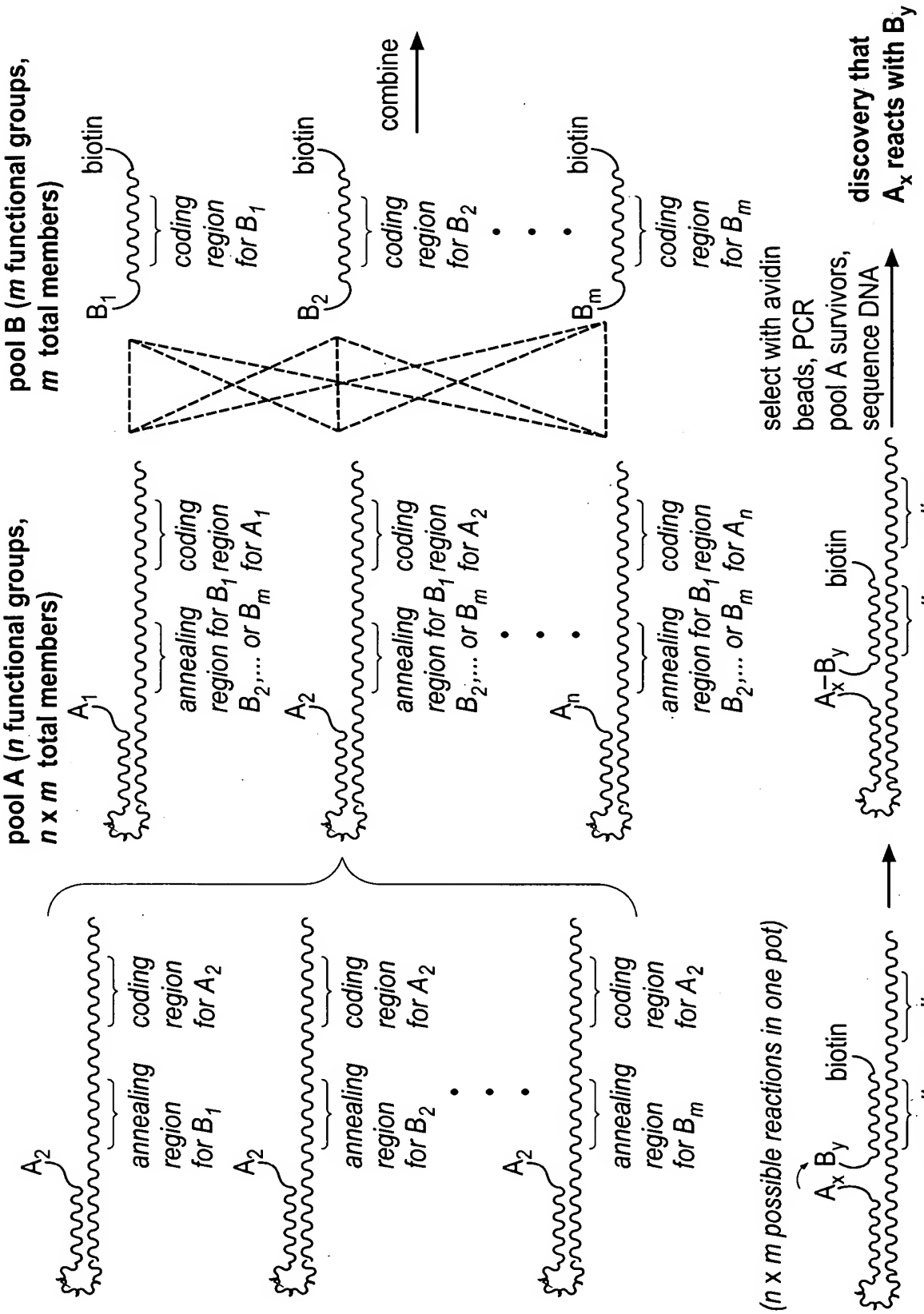

pool A (n functional groups, n x m total members)
pool B (m functional groups, m total members)
biotin
coding region for B1
coding region for B2
coding region for Bm
combine
annealing region for B1 or B2,... or Bm
coding region for A1
coding region for A2
coding region for An
select with avidin beads, PCR
pool A survivors, sequence DNA
discovery that Ax reacts with By
(n x m possible reactions in one pot)
annealing region for By
coding region for Ax
annealing region for Bm
coding region for A2

FIG. 9

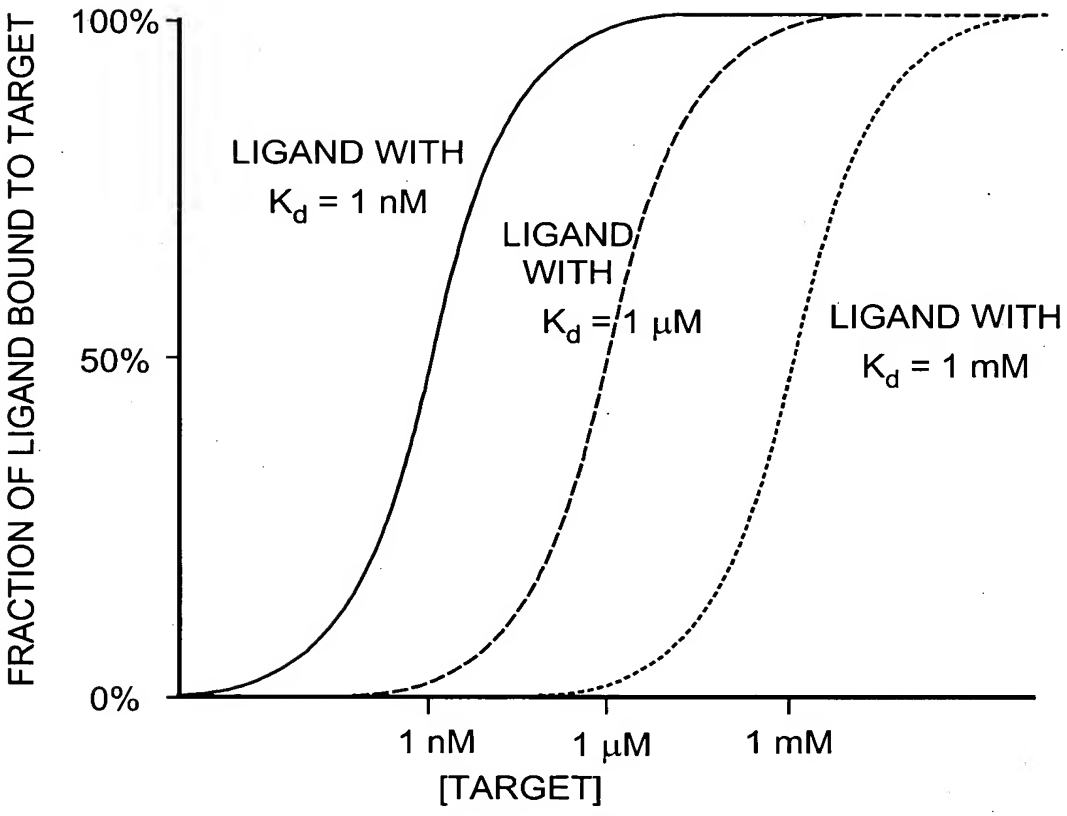

FRACTION OF LIGAND BOUND TO TARGET
100%
50%
0%
LIGAND WITH
K_d = 1 nM
LIGAND WITH
K_d = 1 μM
LIGAND WITH
K_d = 1 mM
1 nM
1 μM
1 mM
[TARGET]

FIG. 10

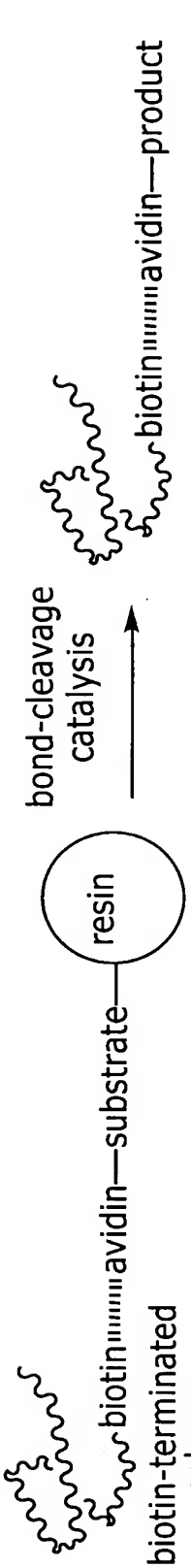


FIG. 11A

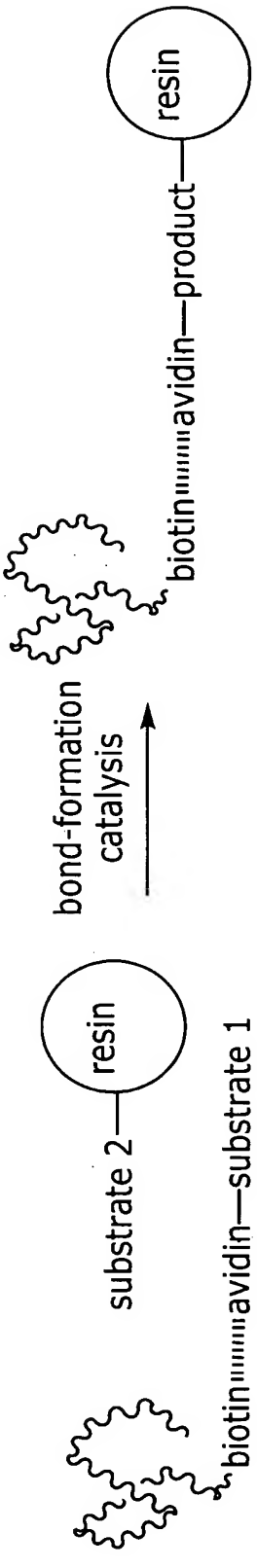


FIG. 11B

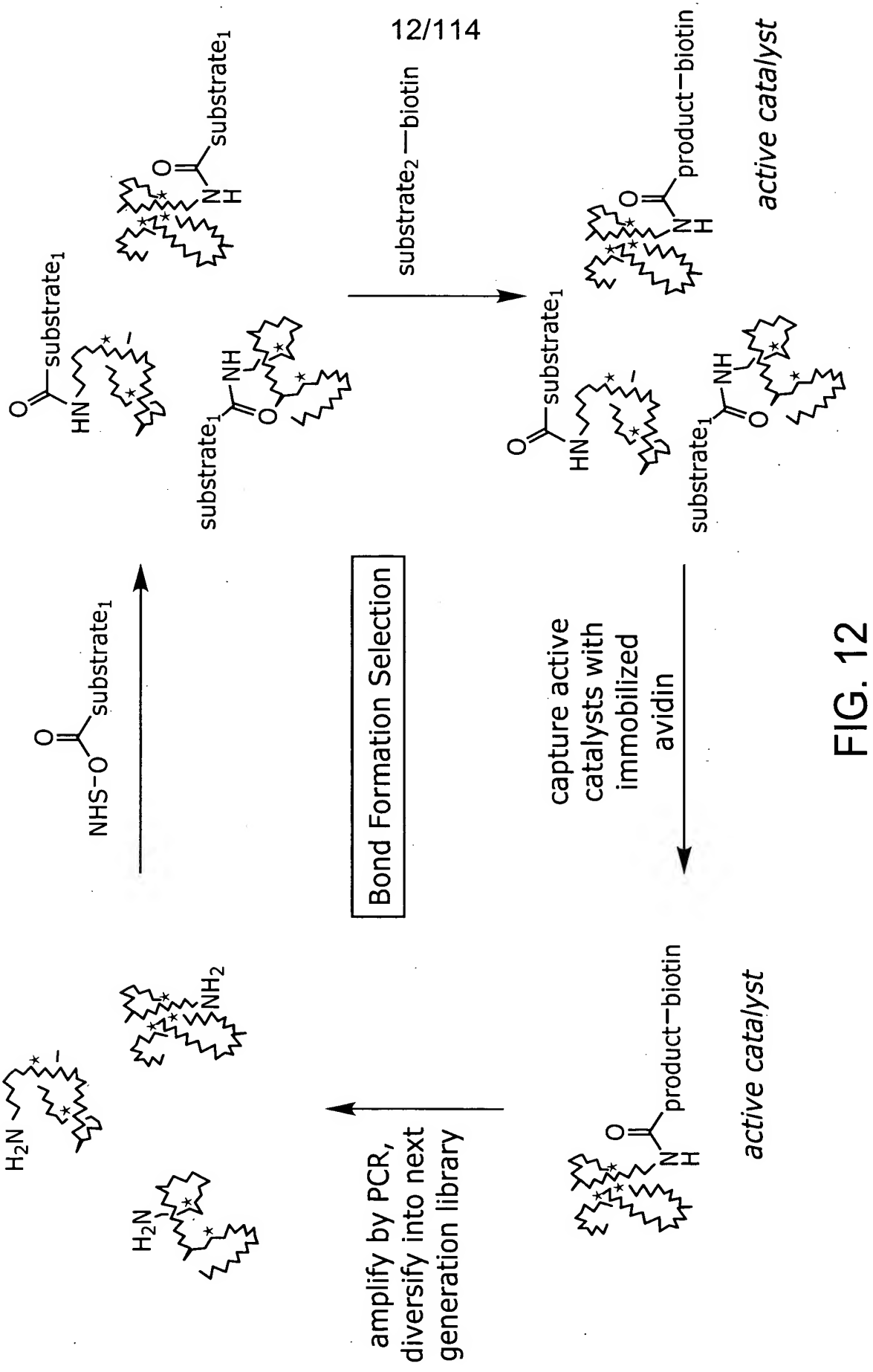

substrate$_1$
substrate$_2$—biotin
product—biotin
active catalyst
Bond Formation Selection
capture active catalysts with immobilized avidin
NHS—O
amplify by PCR, diversify into next generation library
H_2N
NH_2

FIG. 12

Title: Evolving New Molecular Function
Inventor: Liu *et al.*
Serial No.: 10/643,752
Atty Docket No.: LS5-001 (9792/5)
Atty/Agent: Brian A. Fairchild
Express Mail Label No.: EL932952135US
Sheet 13 of 114

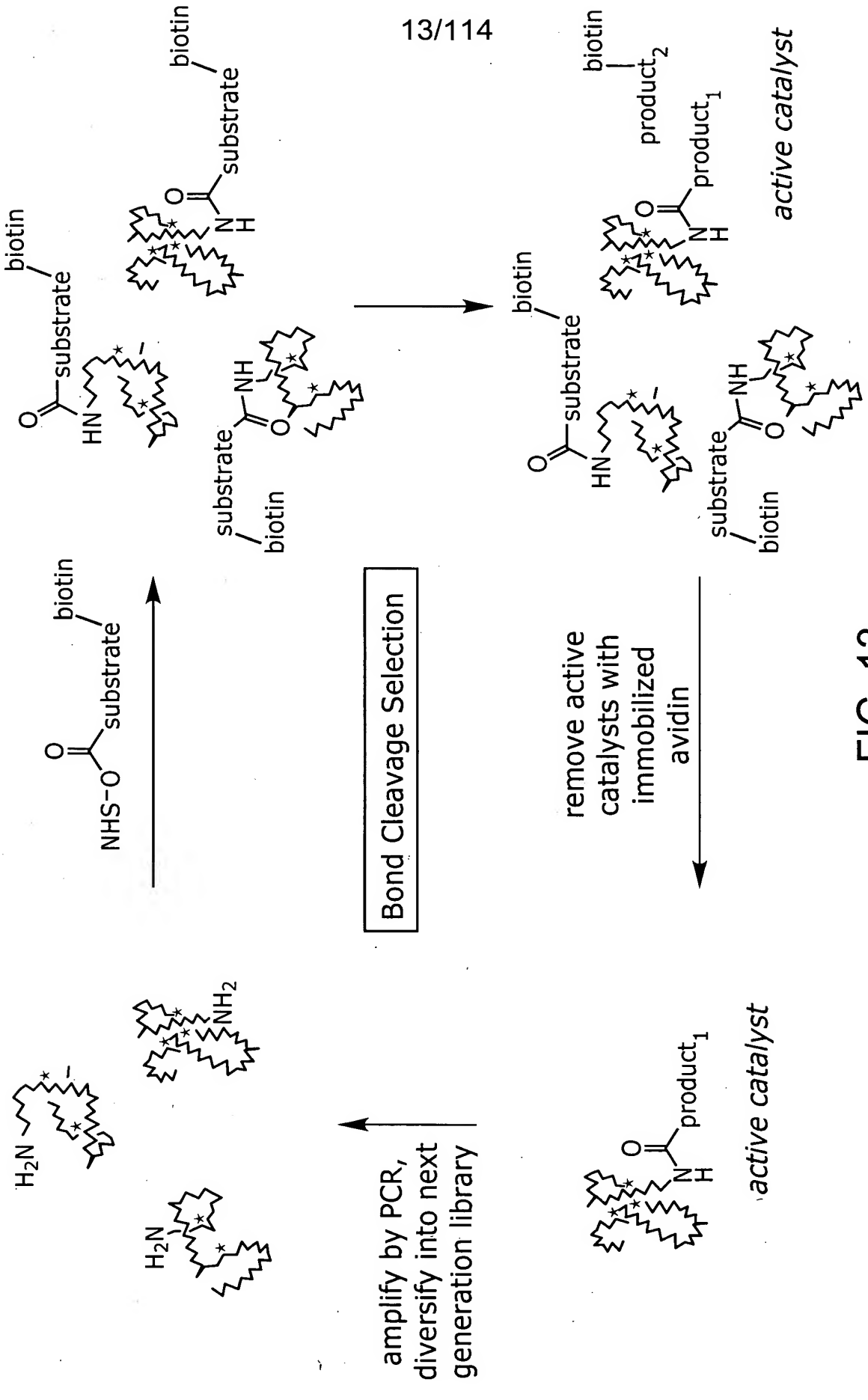


FIG. 13

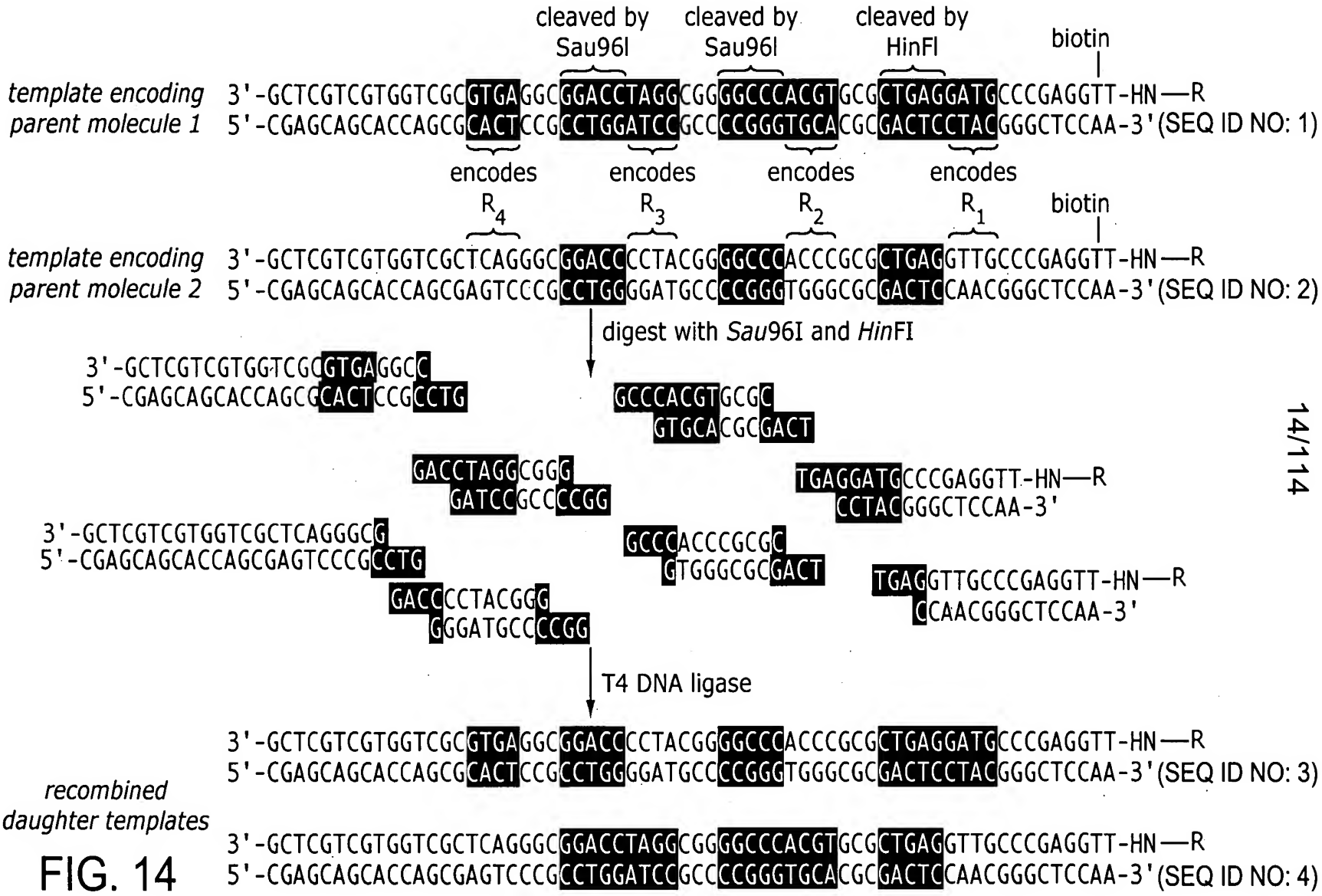


FIG. 14

Title: Evolving New Molecular Function
Inventor: Liu *et al.*
Serial No.: 10/643,752
Atty Docket No.: LS5-001 (9792/5)
Atty/Agent: Brian A. Fairchild
Express Mail Label No.: EL932952135US
Sheet 14 of 114

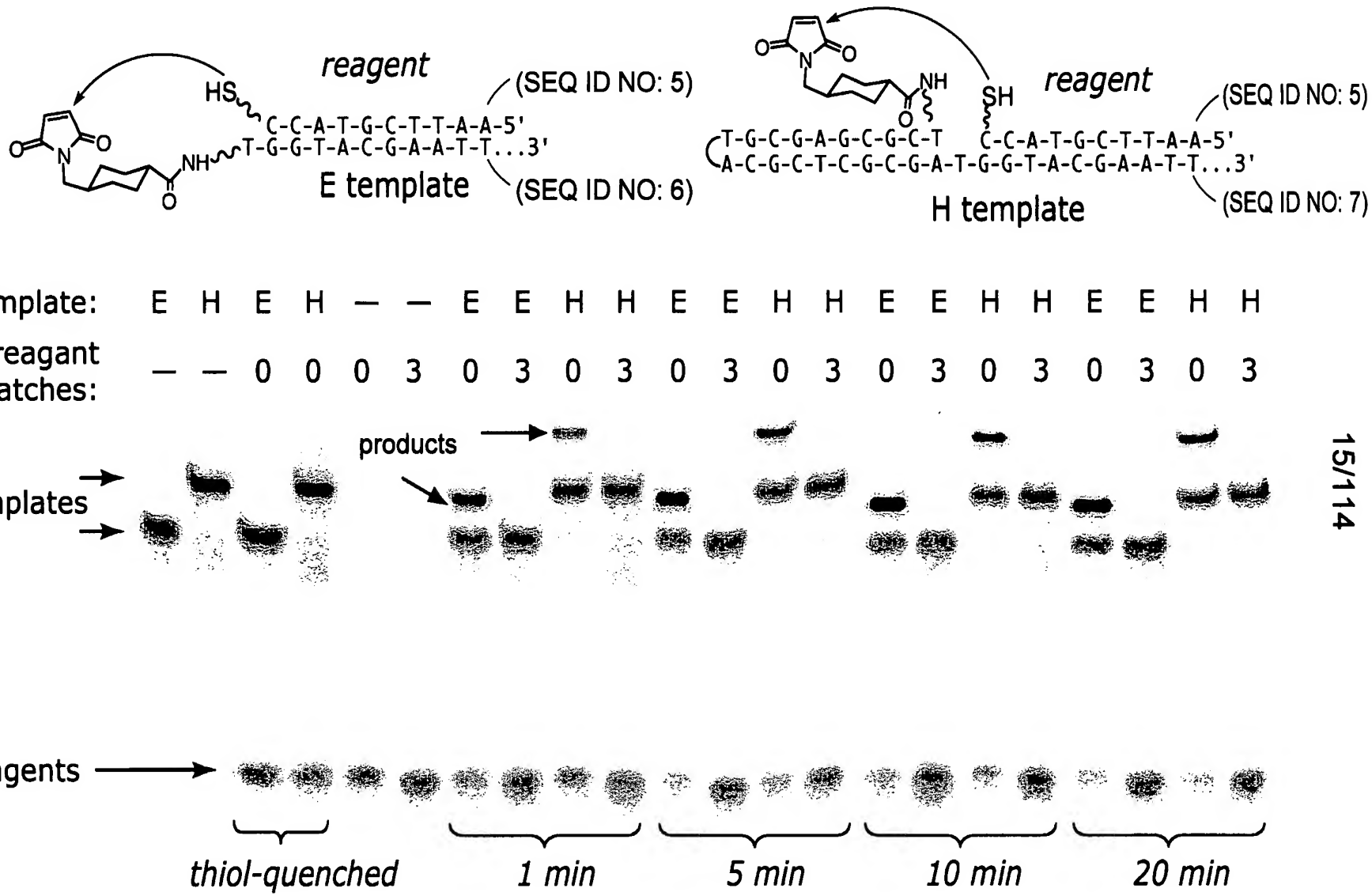


FIG. 15

Title: Evolving New Molecular Function
Inventor: Liu *et al.*
Serial No.: 10/643,752
Atty Docket No.: LS5-001 (9792/5)
Atty/Agent: Brian A. Fairchild
Express Mail Label No.: EL932952135US
Sheet 15 of 114

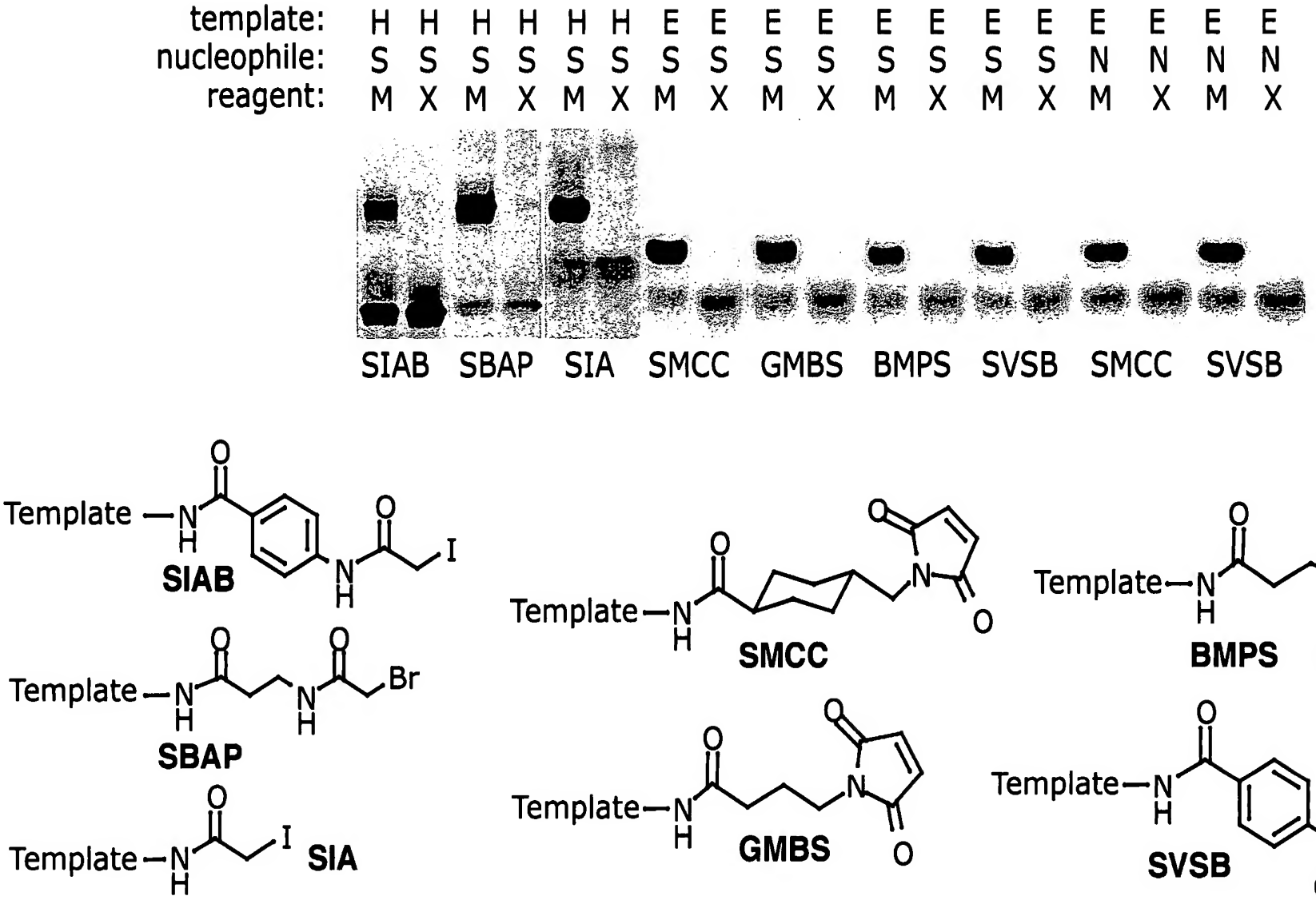

template: H H H H H H E E E E E E E E E E E E
nucleophile: S S S S S S S S S S S S S S N N N N
reagent: M X M X M X M X M X M X M X M X M X
SIAB SBAP SIA SMCC GMBS BMPS SVSB SMCC SVSB
Template
SIAB
Template
SBAP
Br
Template
SIA
I
Template
SMCC
Template
GMBS
Template
BMPS
Template
SVSB

FIG. 16

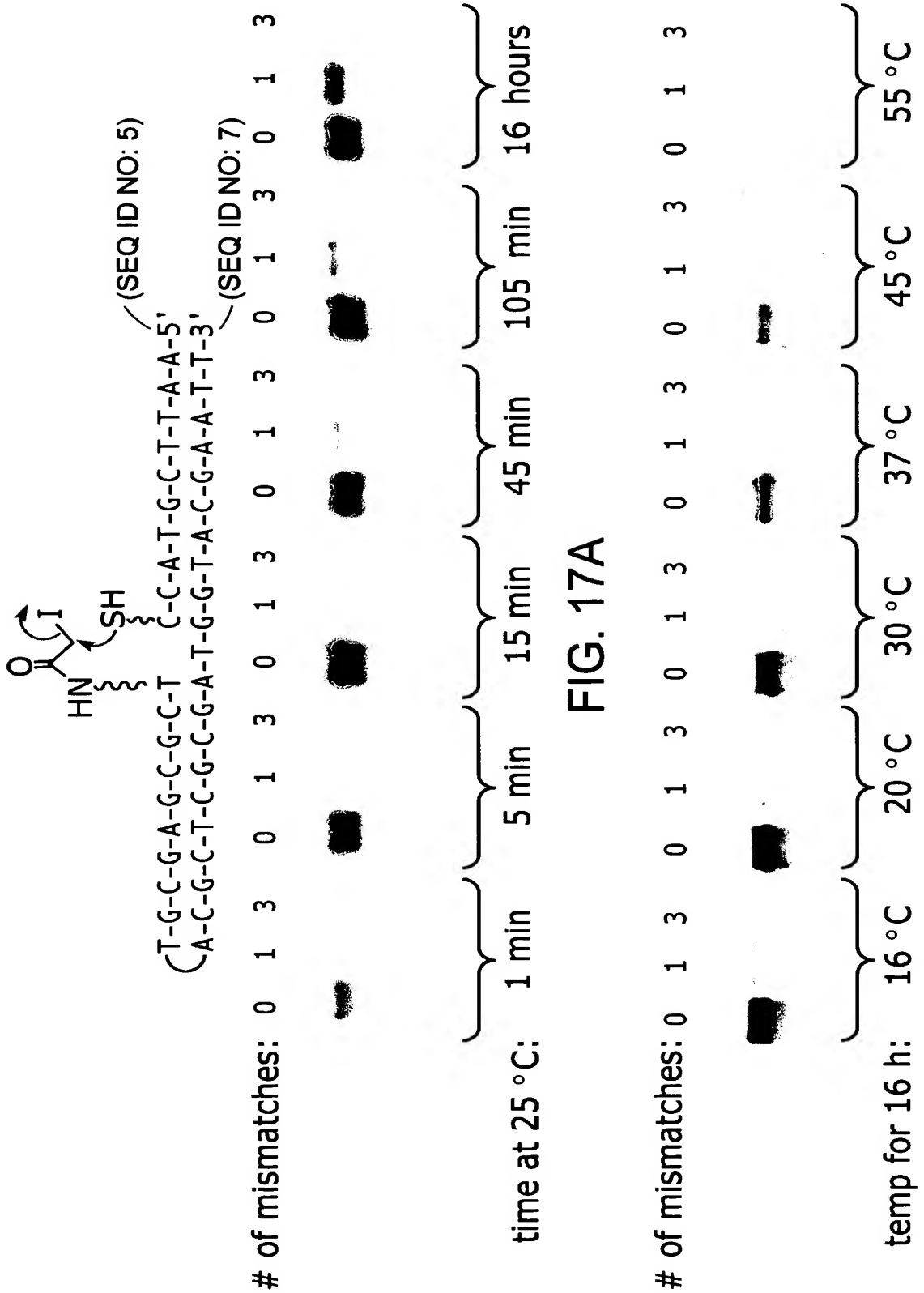
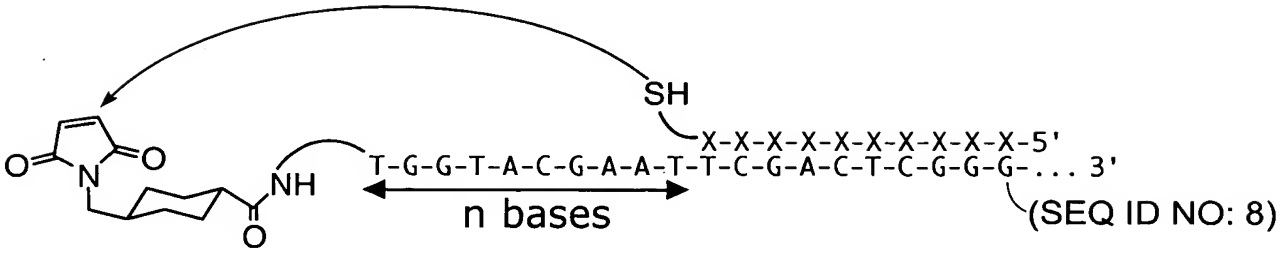


FIG. 17A

FIG. 17B


SH
X-X-X-X-X-X-X-X-X-X-5'
T-G-G-T-A-C-G-A-A-T-T-C-G-A-C-T-C-G-G-G-... 3'
NH
n bases
(SEQ ID NO: 8)

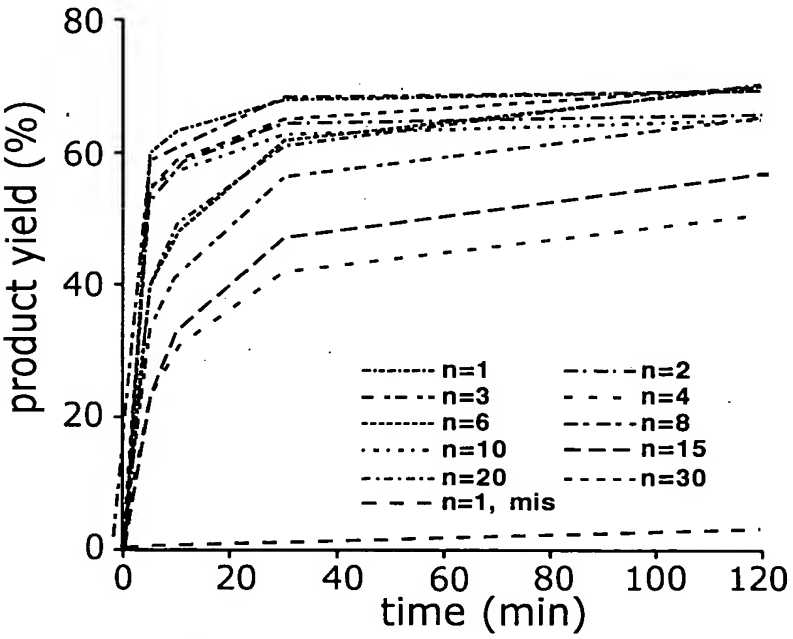

product yield (%)
time (min)
0
20
40
60
80
100
120
n=1
n=2
n=3
n=4
n=6
n=8
n=10
n=15
n=20
n=30
n=1, mis

FIG. 18

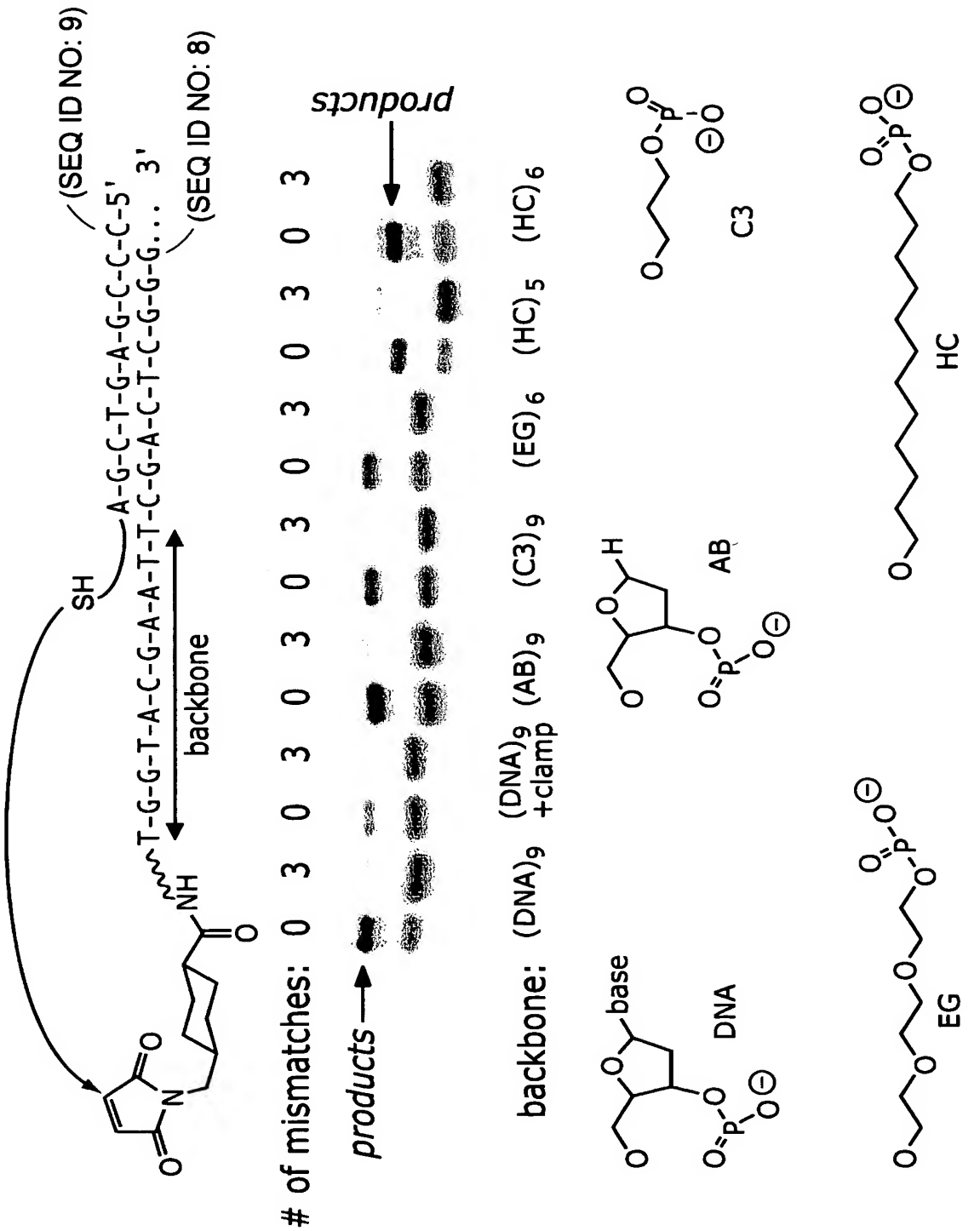

(SEQ ID NO: 9)
SH
A-G-C-T-G-A-G-C-C-C-5'
3'
(SEQ ID NO: 8)
T-G-G-T-A-C-G-A-A-T-T-C-G-A-C-T-C-G-G-G...
backbone
products
# of mismatches: 0 3 0 3 0 3 0 3 0 3 0 3 0 3 0 3
backbone: (DNA)9 (DNA)9 +clamp (AB)9 (C3)9 (EG)6 (HC)5 (HC)6
base
DNA
AB
C3
EG
HC

FIG. 19

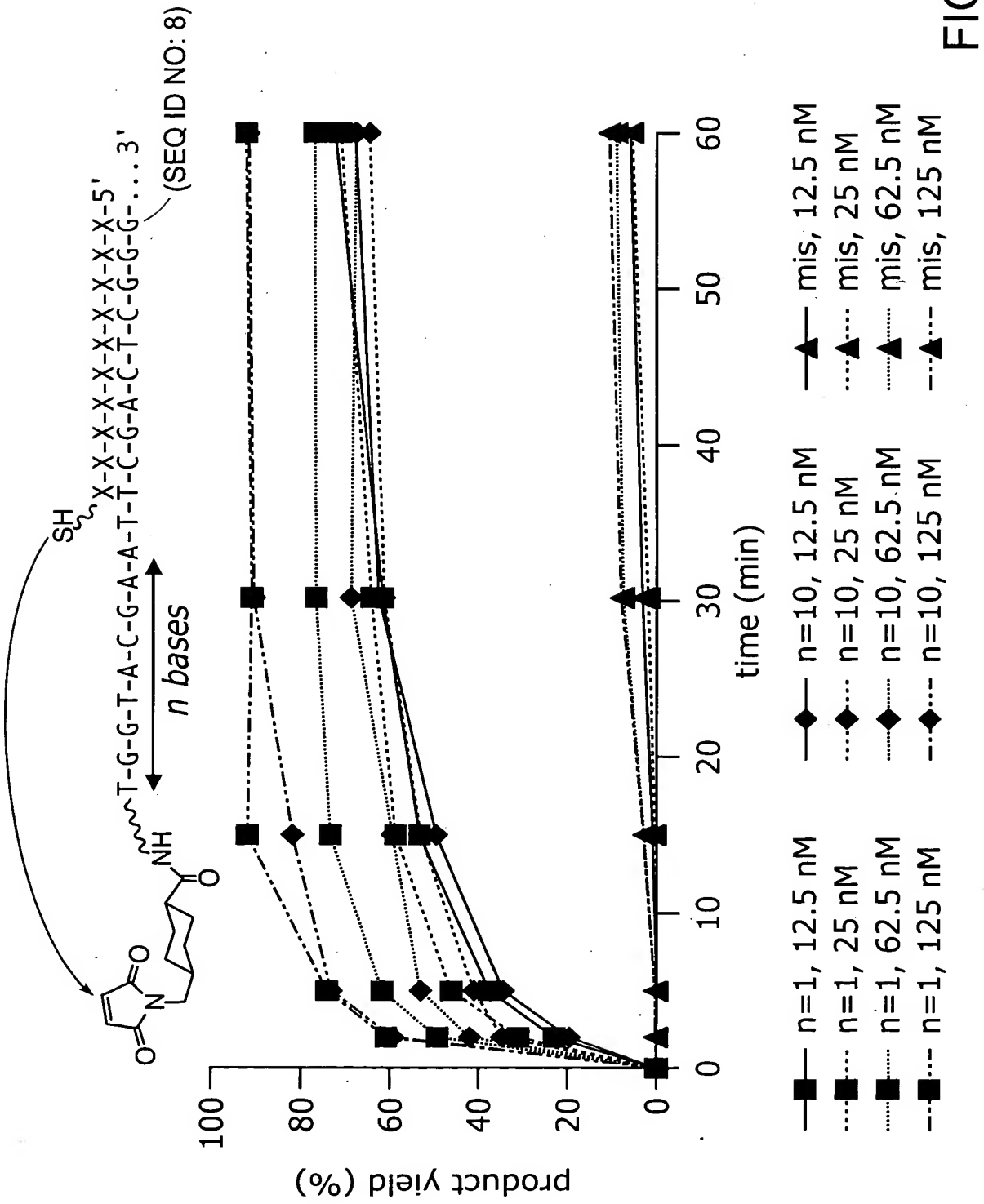

SH
X-X-X-X-X-X-X-X-X-X-X-5'
(SEQ ID NO: 8)
T-G-G-T-A-C-G-A-A-T-T-C-G-A-C-T-C-G-G-G-...3'
n bases
NH
O
O
N
O
O
product yield (%)
100
80
60
40
20
0
0
10
20
30
40
50
60
time (min)
n=1, 12.5 nM
n=1, 25 nM
n=1, 62.5 nM
n=1, 125 nM
n=10, 12.5 nM
n=10, 25 nM
n=10, 62.5 nM
n=10, 125 nM
mis, 12.5 nM
mis, 25 nM
mis, 62.5 nM
mis, 125 nM

FIG. 20

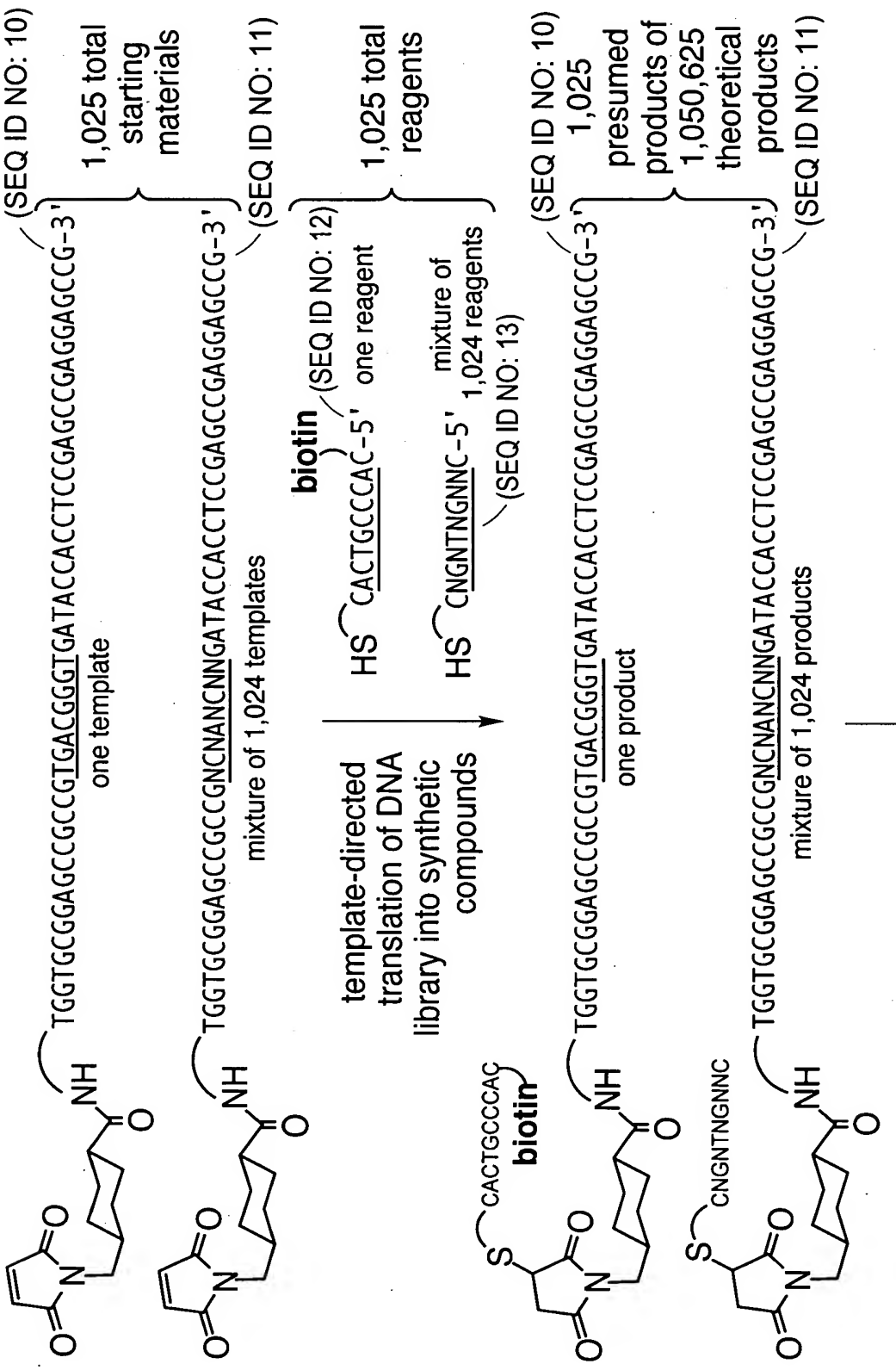

TGGTGCGGAGCCGCCGTGACGGGTGATACCACCTCCGAGCCGAGGAGCCG-3'
(SEQ ID NO: 10)
one template
1,025 total starting materials
TGGTGCGGAGCCGCCGNCNANCNNGATACCACCTCCGAGCCGAGGAGCCG-3'
mixture of 1,024 templates
(SEQ ID NO: 11)
template-directed translation of DNA library into synthetic compounds
HS CACTGCCCAC-5'
biotin
(SEQ ID NO: 12)
one reagent
HS CNGNTNGNNC-5'
mixture of 1,024 reagents
(SEQ ID NO: 13)
1,025 total reagents
CACTGCCCAC
biotin
TGGTGCGGAGCCGCCGTGACGGGTGATACCACCTCCGAGCCGAGGAGCCG-3'
(SEQ ID NO: 10)
one product
1,025 presumed products of 1,050,625 theoretical products
CNGNTNGNNC
TGGTGCGGAGCCGCCGNCNANCNNGATACCACCTCCGAGCCGAGGAGCCG-3'
mixture of 1,024 products
(SEQ ID NO: 11)

FIG. 21A

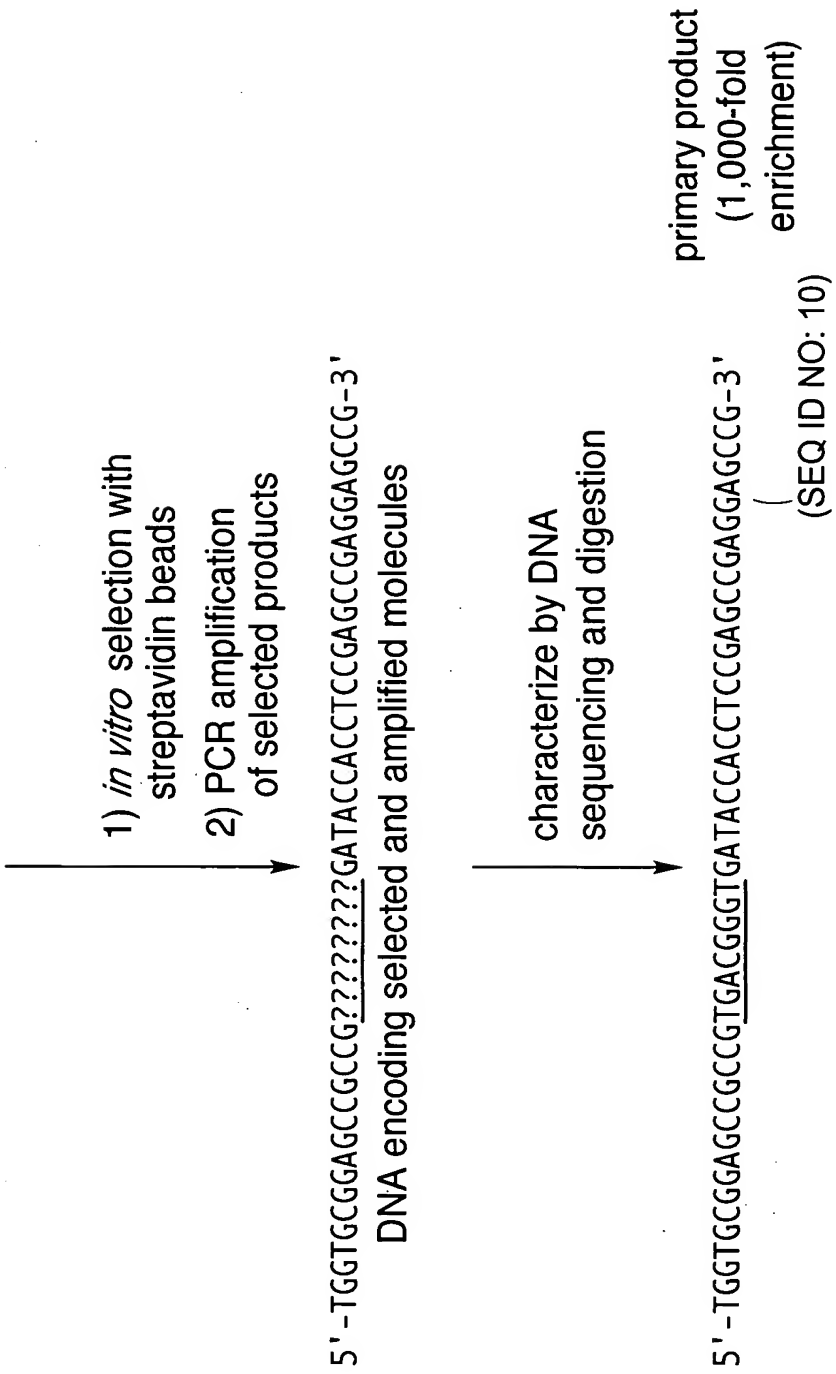
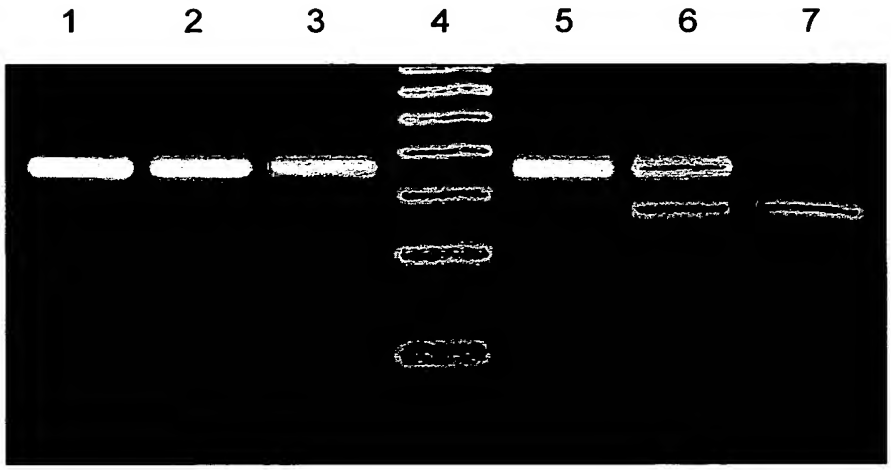
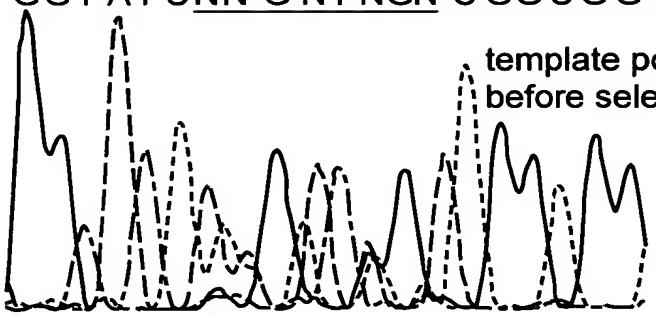

1) *in vitro* selection with streptavidin beads
2) PCR amplification of selected products
5'-TGGTGCGGAGCCGCCG??????GATACCACCTCCGAGCCGAGGAGCCG-3'
DNA encoding selected and amplified molecules
characterize by DNA sequencing and digestion
5'-TGGTGCGGAGCCGCCGTGACGGGTGATACCACCTCCGAGCCGAGGAGCCG-3'
(SEQ ID NO: 10)
primary product (1,000-fold enrichment)

FIG. 21B



3'---GGT AT CNN G NT NGN C GGC G G--- non-biotin encoding (residues 30-11 of SEQ ID NO: 11)



3'---GGT AT CAC C CGT CACGG C GG--- biotin encoding (residues 30-11 of SEQ ID NO: 10)

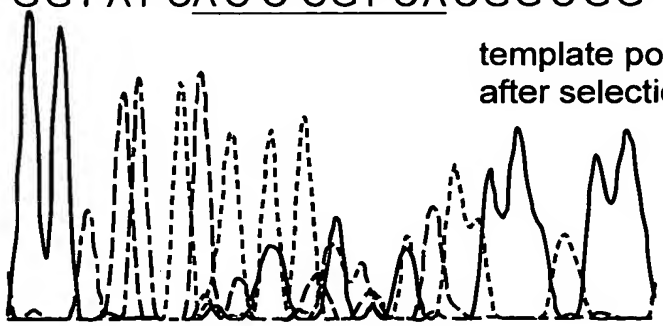


FIG. 22A

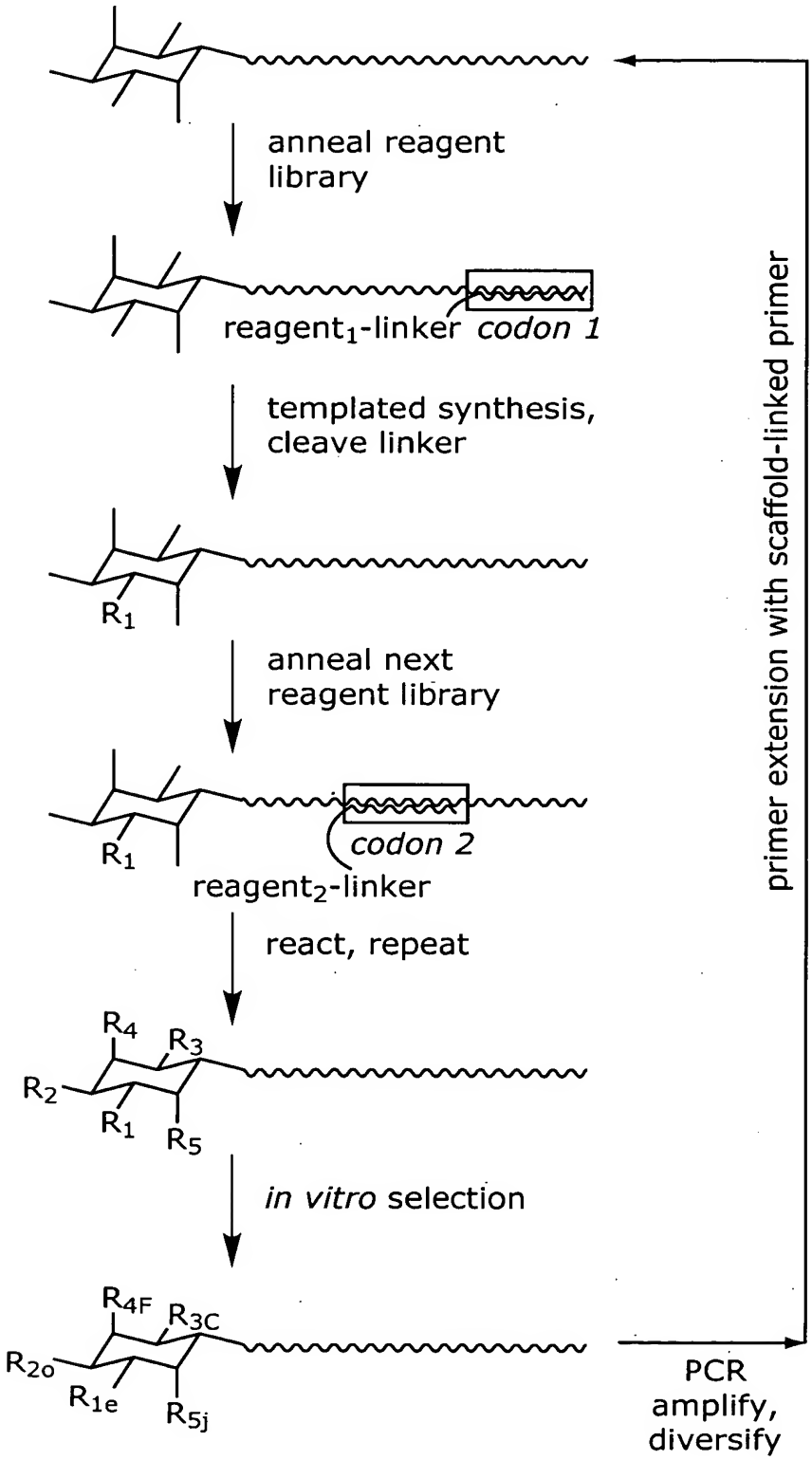
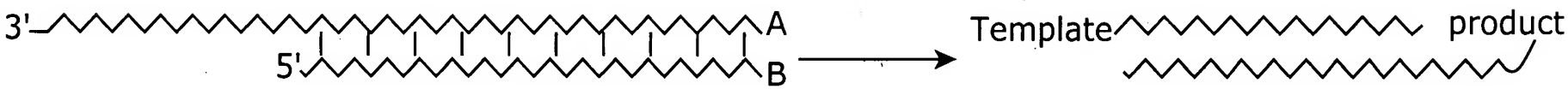

anneal reagent library
reagent1-linker codon 1
templated synthesis, cleave linker
R1
anneal next reagent library
codon 2
reagent2-linker
react, repeat
R4
R3
R2
R1
R5
in vitro selection
R4F
R3C
R2o
R1e
R5j
PCR amplify, diversify
primer extension with scaffold-linked primer

FIG. 22B



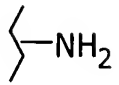
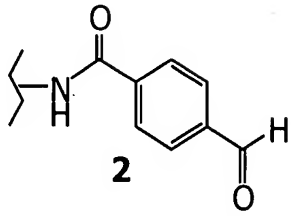
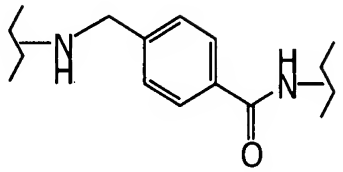
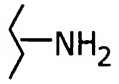
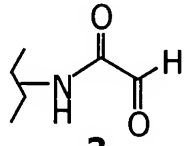
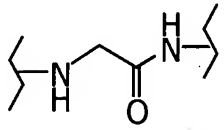
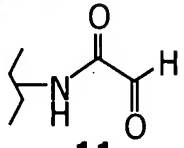
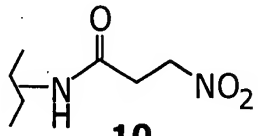
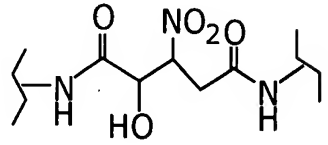
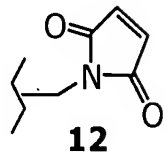
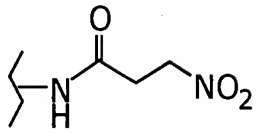
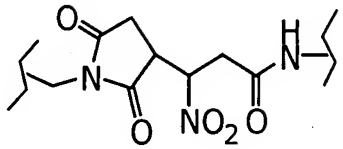
A	B	conditions	product	yield (%)
 **1**	 **2**	a		81
 **1**	 **3**	a		70
 **11**	 **10**	b		45
 **12**	 **10**	b		42

FIG. 23A

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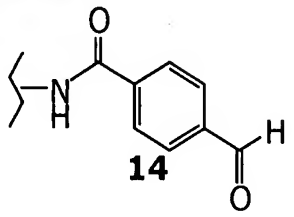
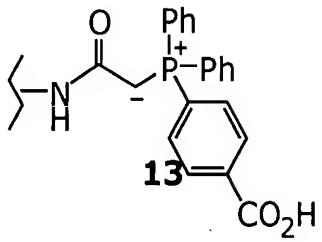
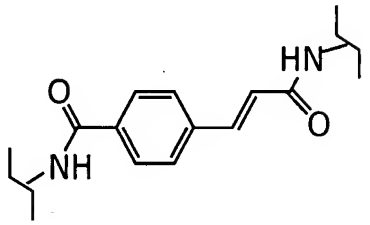
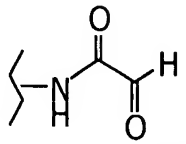
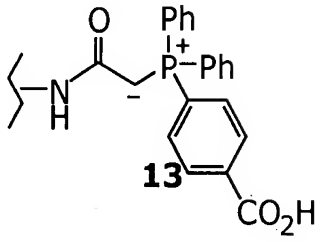
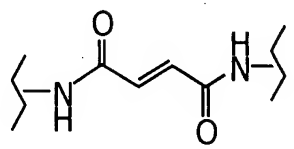
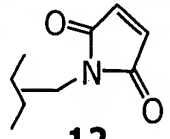
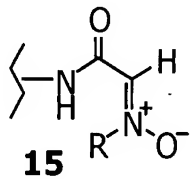
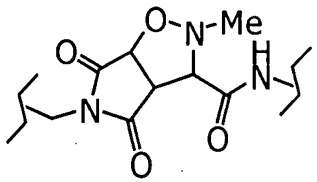
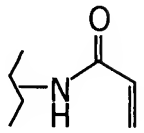
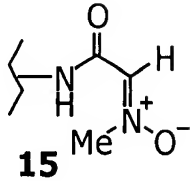
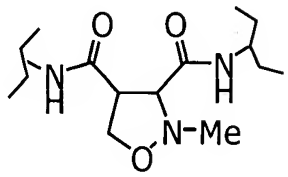
A	B	conditions	product	yield (%)
 **14**	 **13**	c		93
 **11**	 **13**	c		>97
 **12**	 **15**	d		53 (R=Me) 42 (R=Bn)
 **17**	 **15**	d		54

FIG. 23B

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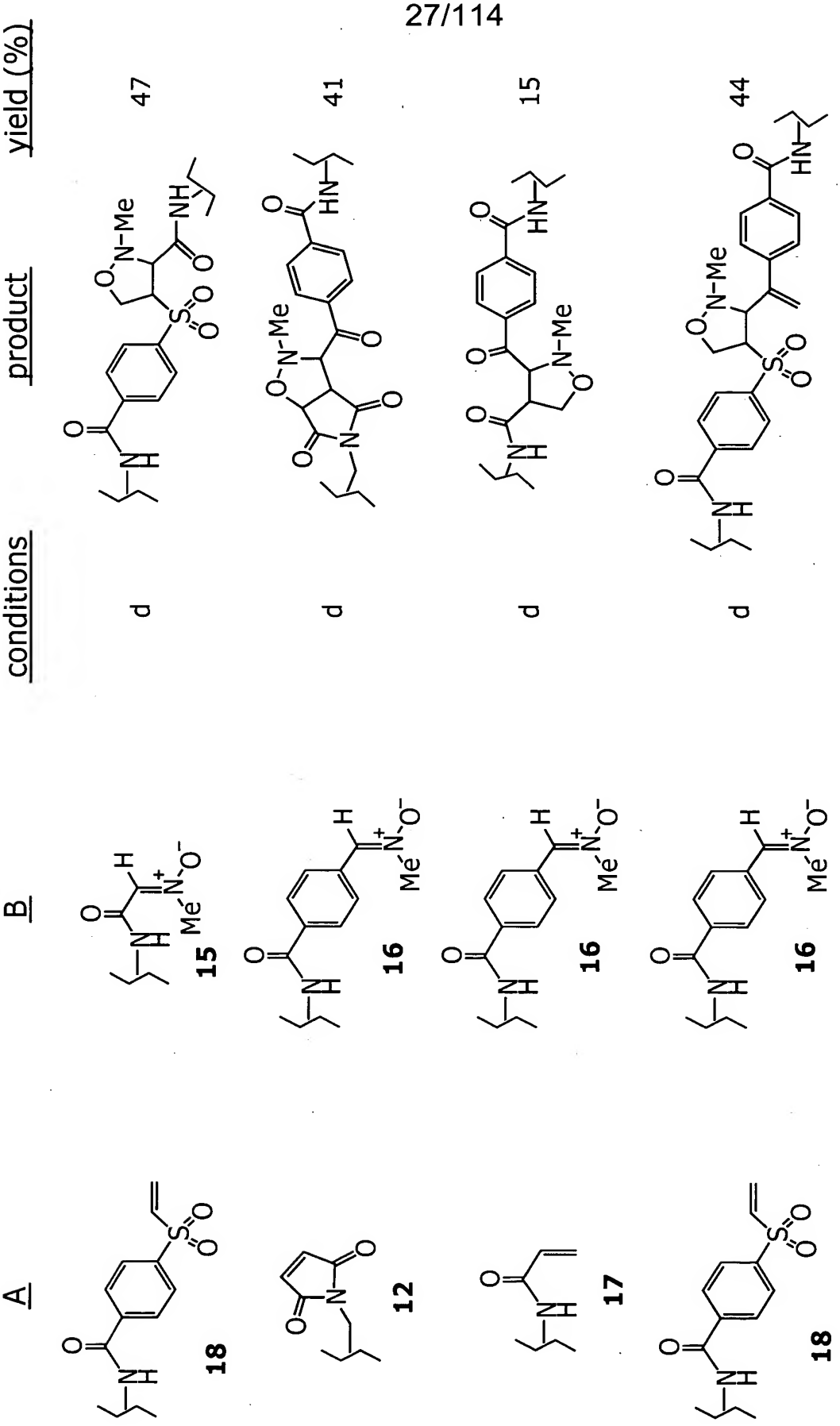

A
B
conditions
product
yield (%)
18
12
17
18
15
16
16
16
d
d
d
d
47
41
15
44

FIG. 23C

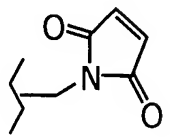
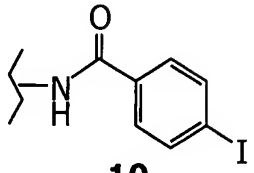
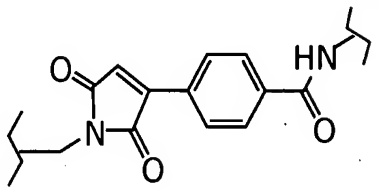
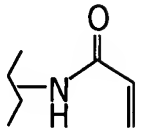
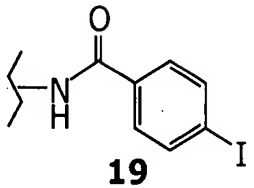
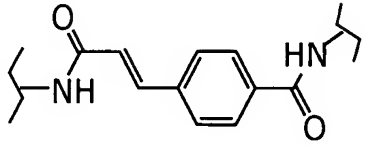
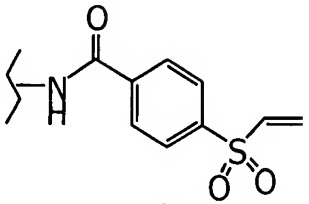
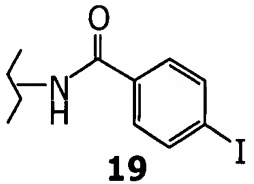
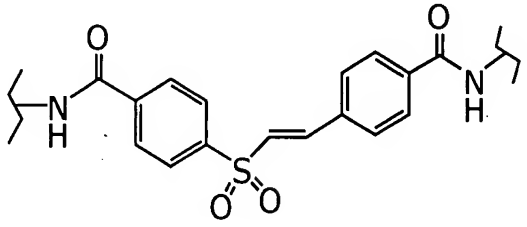
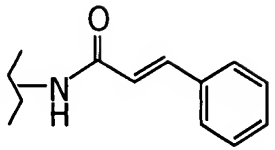
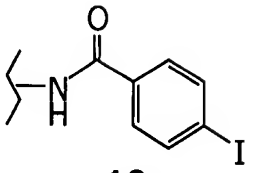
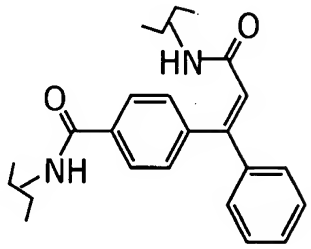
A	B	conditions	product	yield (%)
 **12**	 **19**	e		54
 **17**	 **19**	f		26
 **18**	 **19**	f		51
 **20**	 **19**	f		31

FIG. 23D

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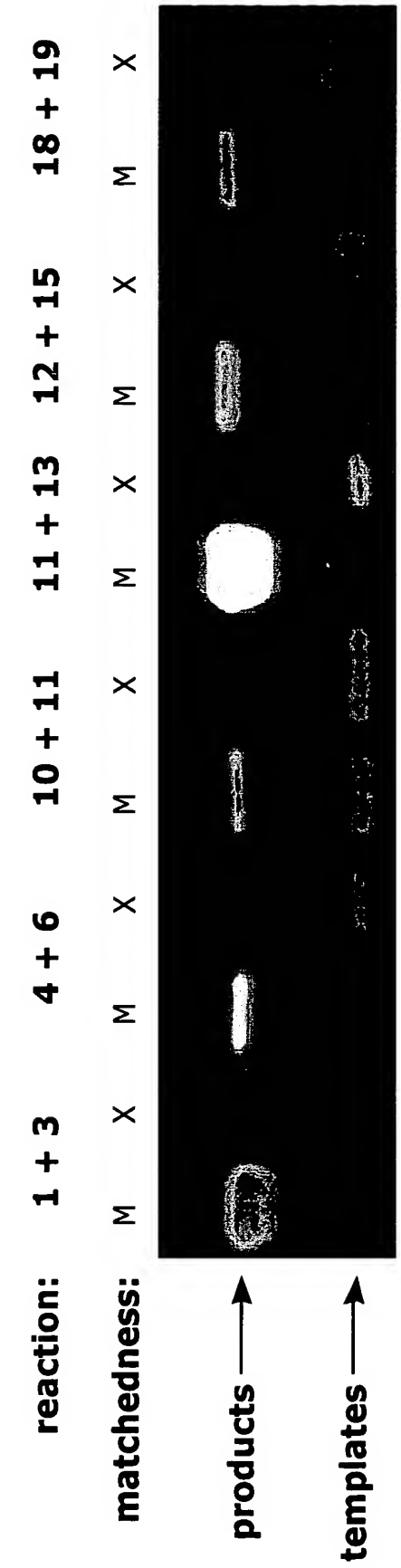
reaction:
1 + 3
4 + 6
10 + 11
11 + 13
12 + 15
18 + 19
matchedness:
M
X
M
X
M
X
M
X
M
X
M
X
products
templates

FIG. 24

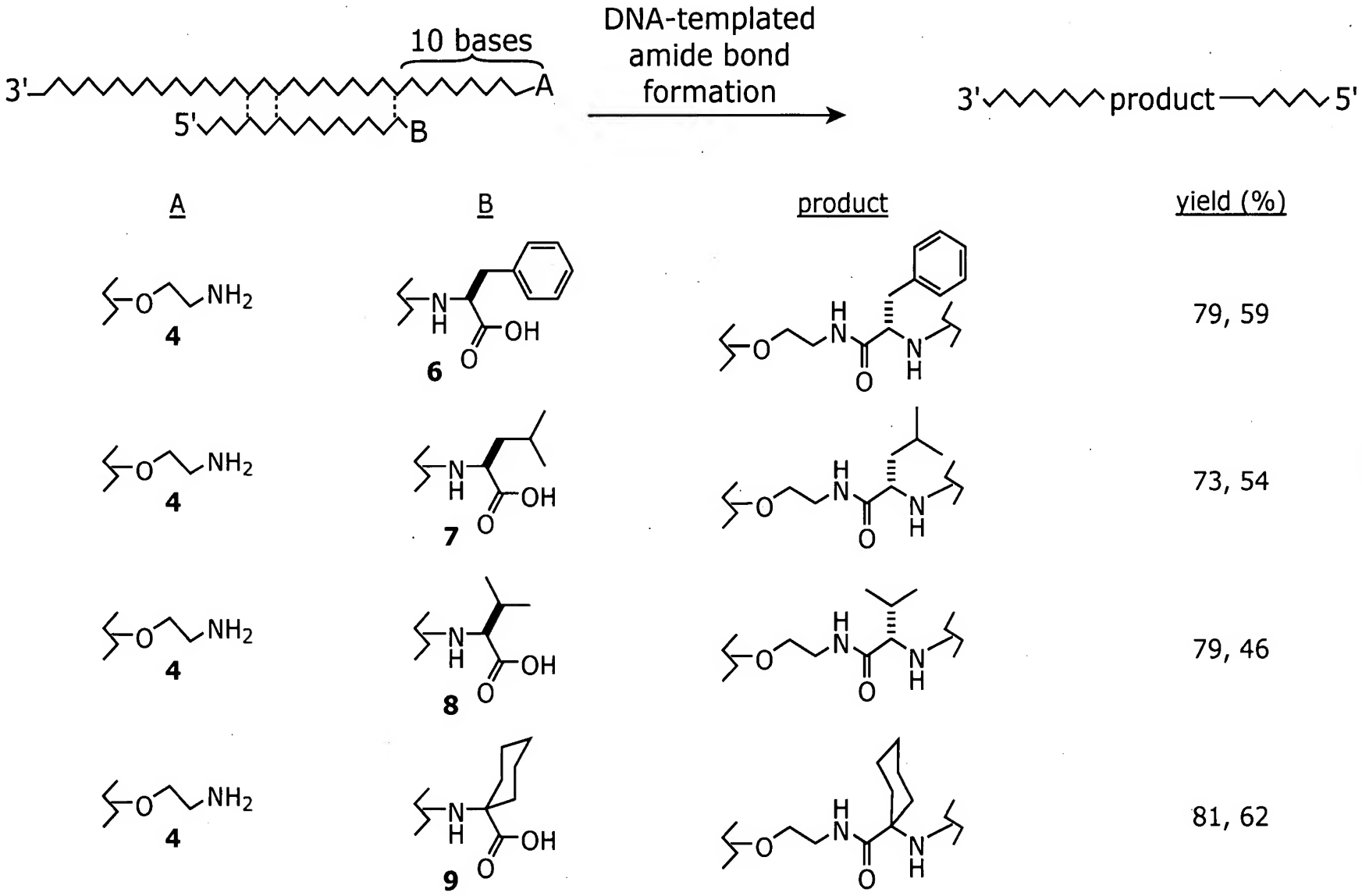

FIG. 25A

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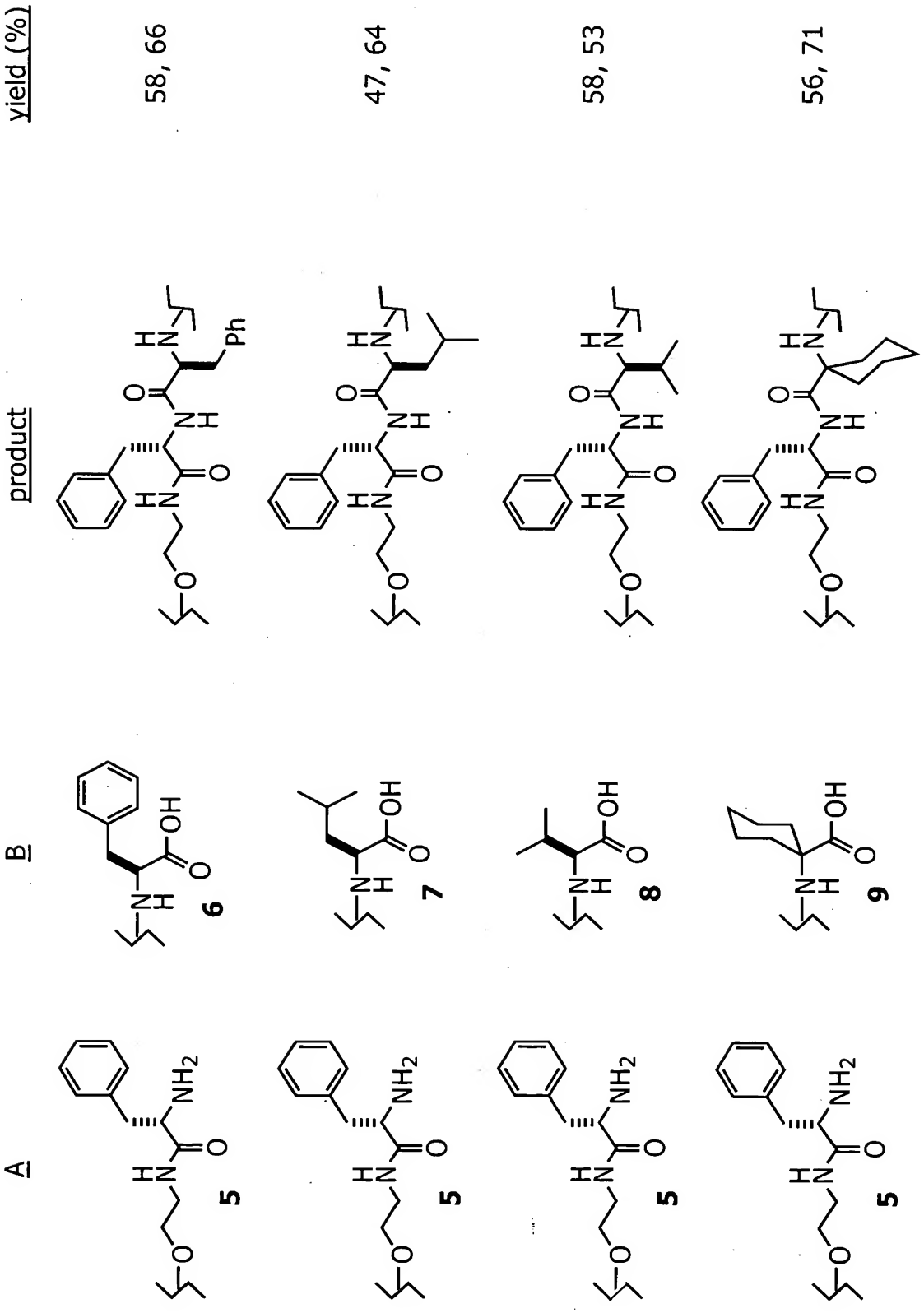


FIG. 25B

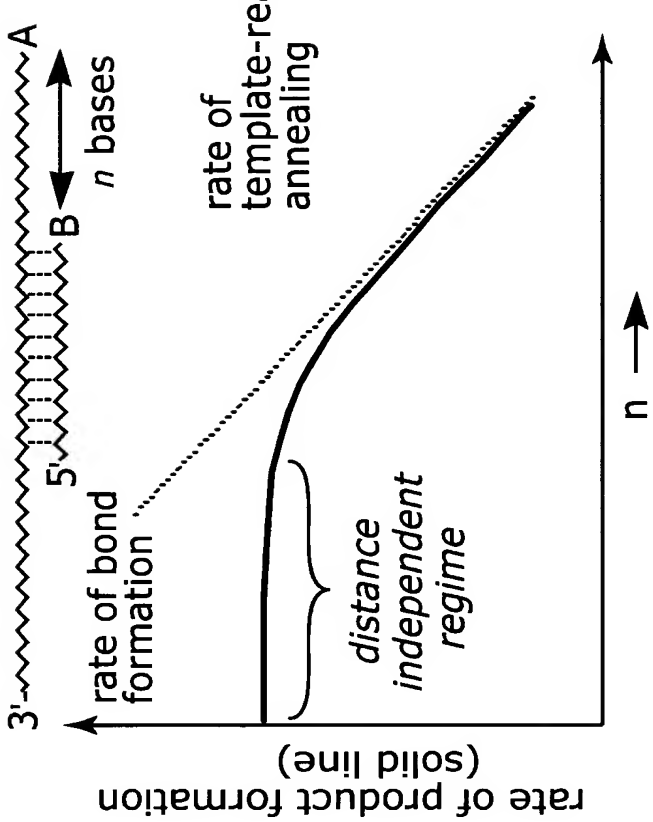


FIG. 26A

FIG. 26B

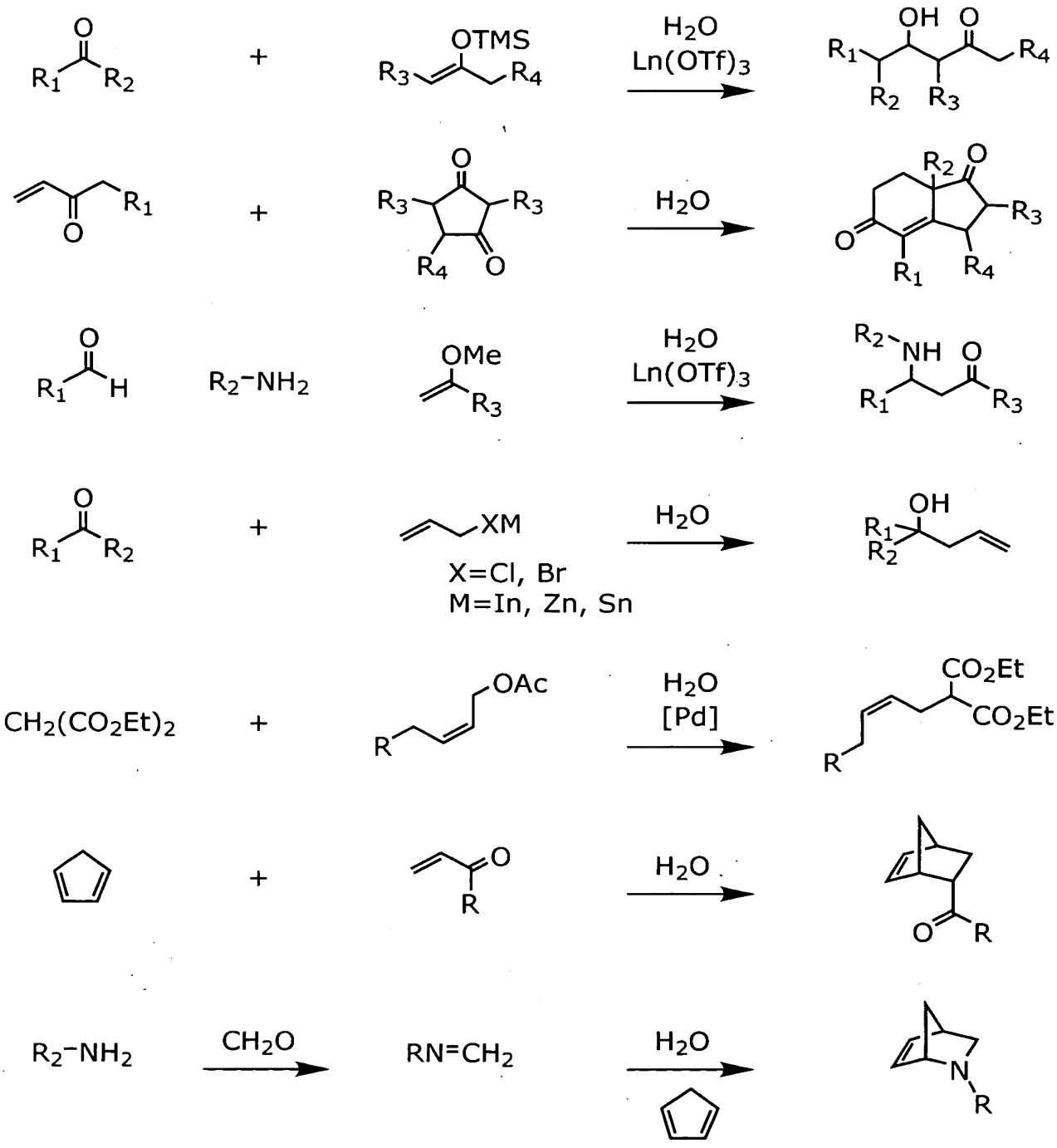

H2O
Ln(OTf)3
OTMS
OH
H2O
[Pd]
CO2Et
CO2Et
CH2(CO2Et)2
OAc
OMe
XM
X=Cl, Br
M=In, Zn, Sn
R2-NH2
CH2O
RN=CH2

FIG. 27

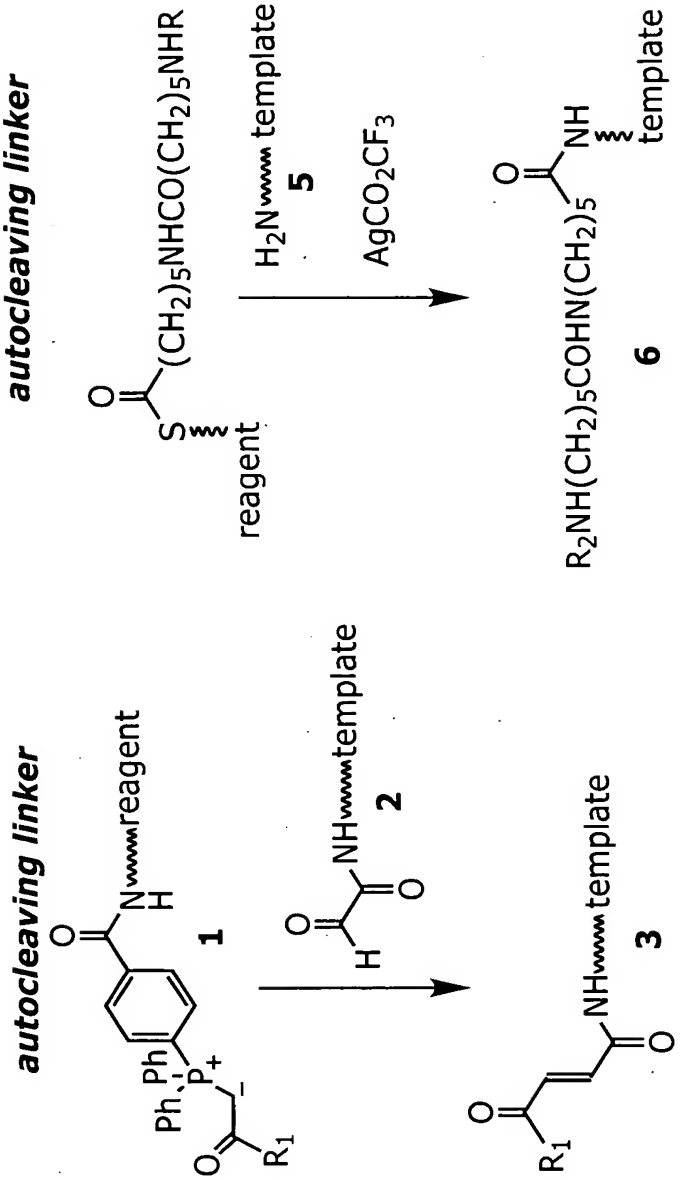


FIG. 28A

FIG. 28B

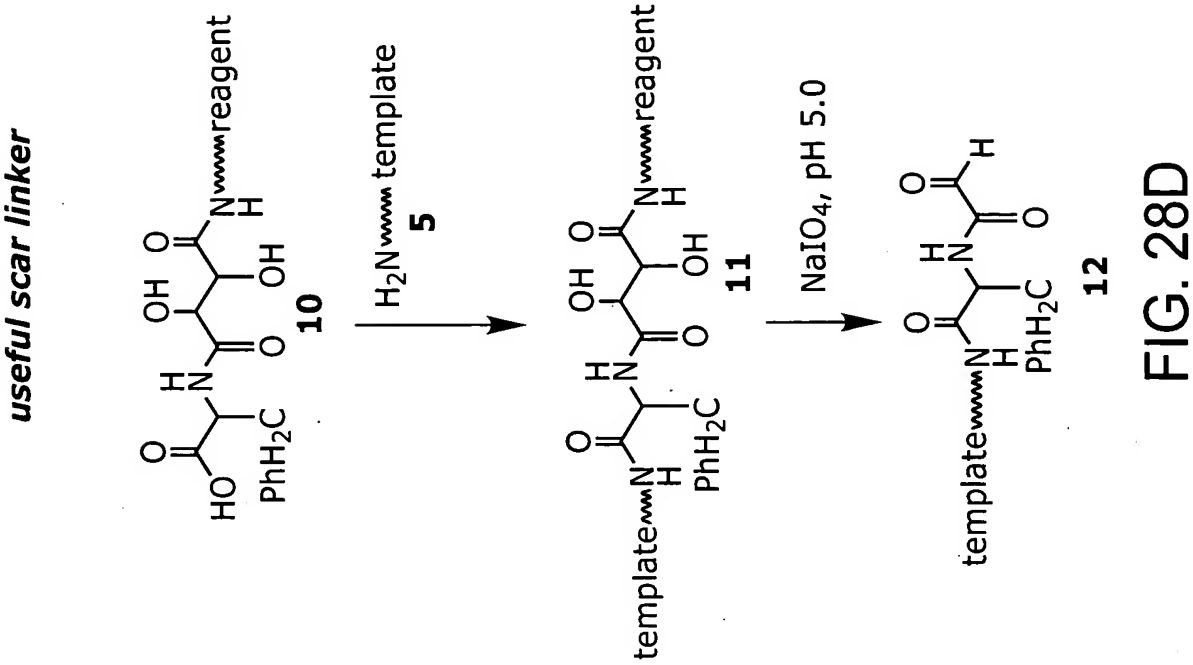


FIG. 28D

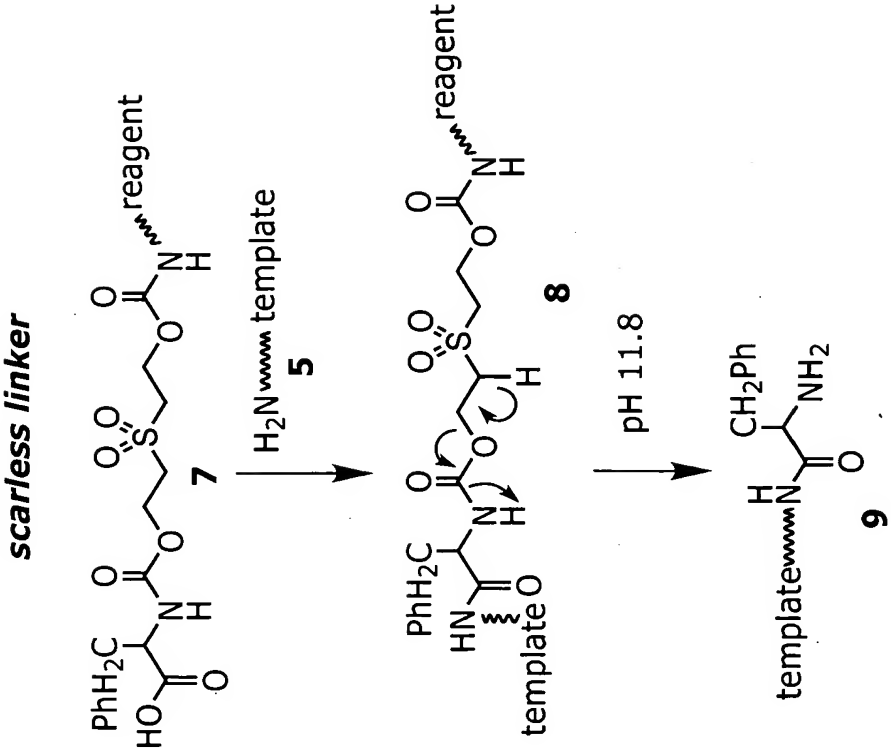


FIG. 28C

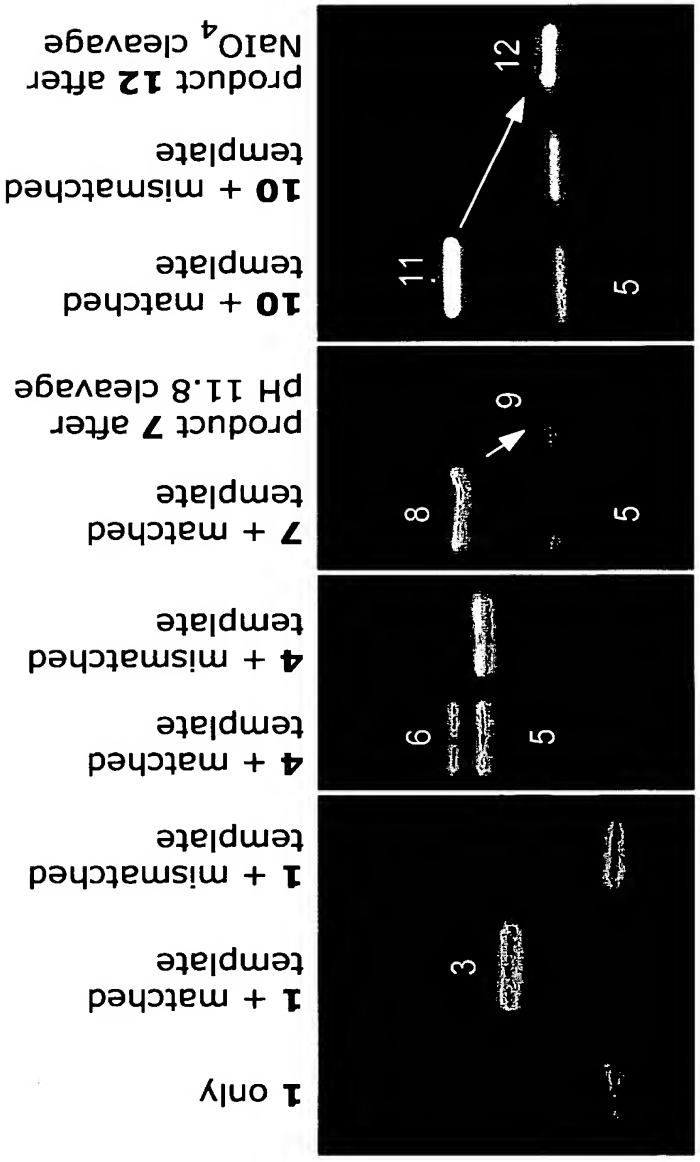

1 only
1 + matched template
1 + mismatched template
4 + matched template
4 + mismatched template
7 + matched template
product 7 after pH 11.8 cleavage
10 + matched template
10 + mismatched template
product 12 after NaIO4 cleavage
3
6
5
8
9
5
11
12
5
unstained (dansylated species only are visible)
stained with ethidium bromide

FIG. 29

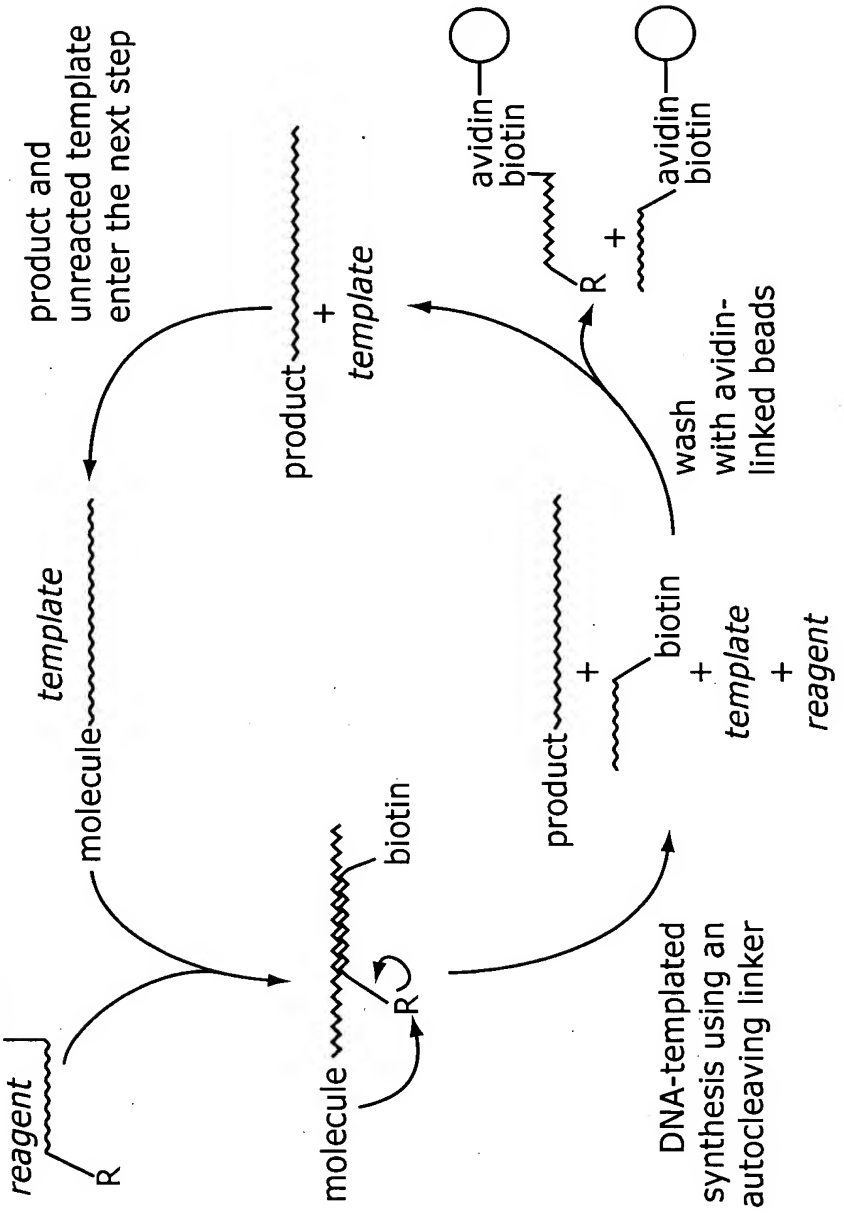

biotin
reagent
R
molecule
template
molecule
R
biotin
DNA-templated synthesis using an autocleaving linker
product
+
biotin
+
template
+
reagent
wash with avidin-linked beads
avidin
biotin
R
+
avidin
biotin
product
+
template
product and unreacted template enter the next step

FIG. 30A

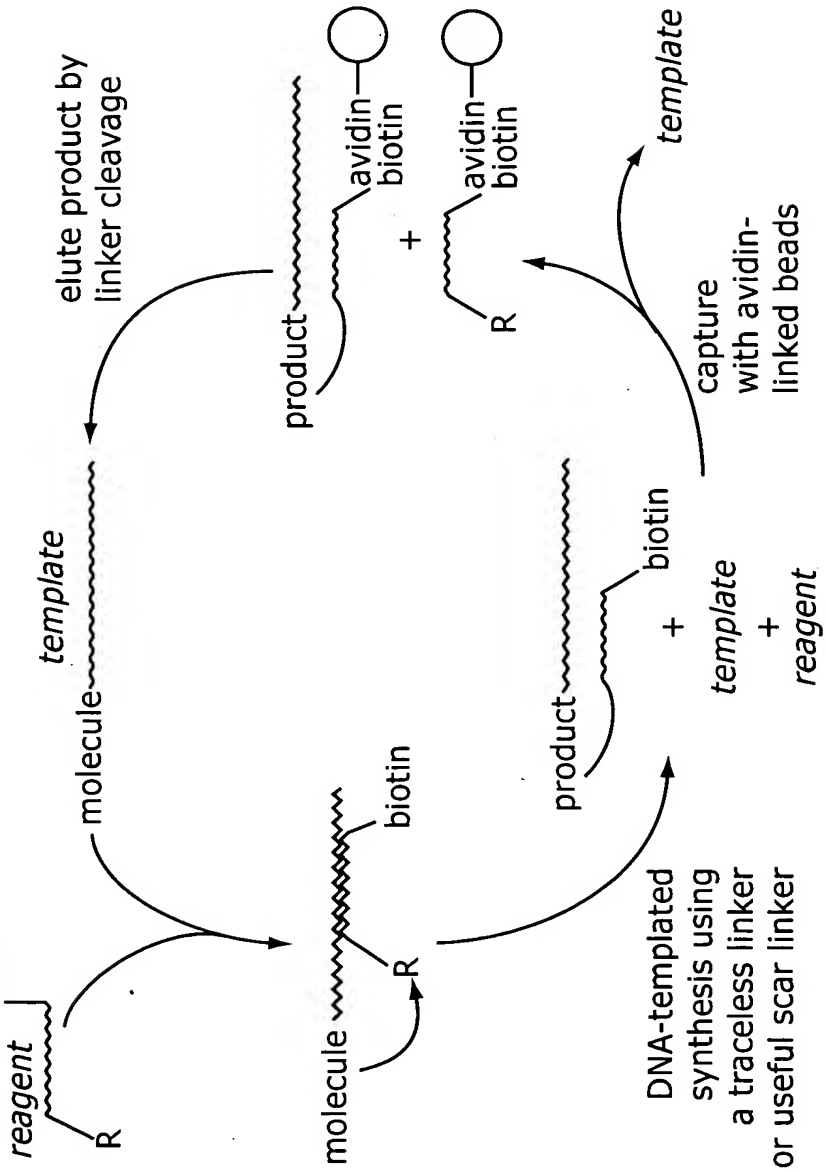

biotin
reagent
R
molecule
R
biotin
DNA-templated synthesis using a traceless linker or useful scar linker
product
biotin
+
template
+
reagent
capture with avidin-linked beads
template
product
avidin
biotin
+
avidin
biotin
R
elute product by linker cleavage
template
molecule

FIG. 30B

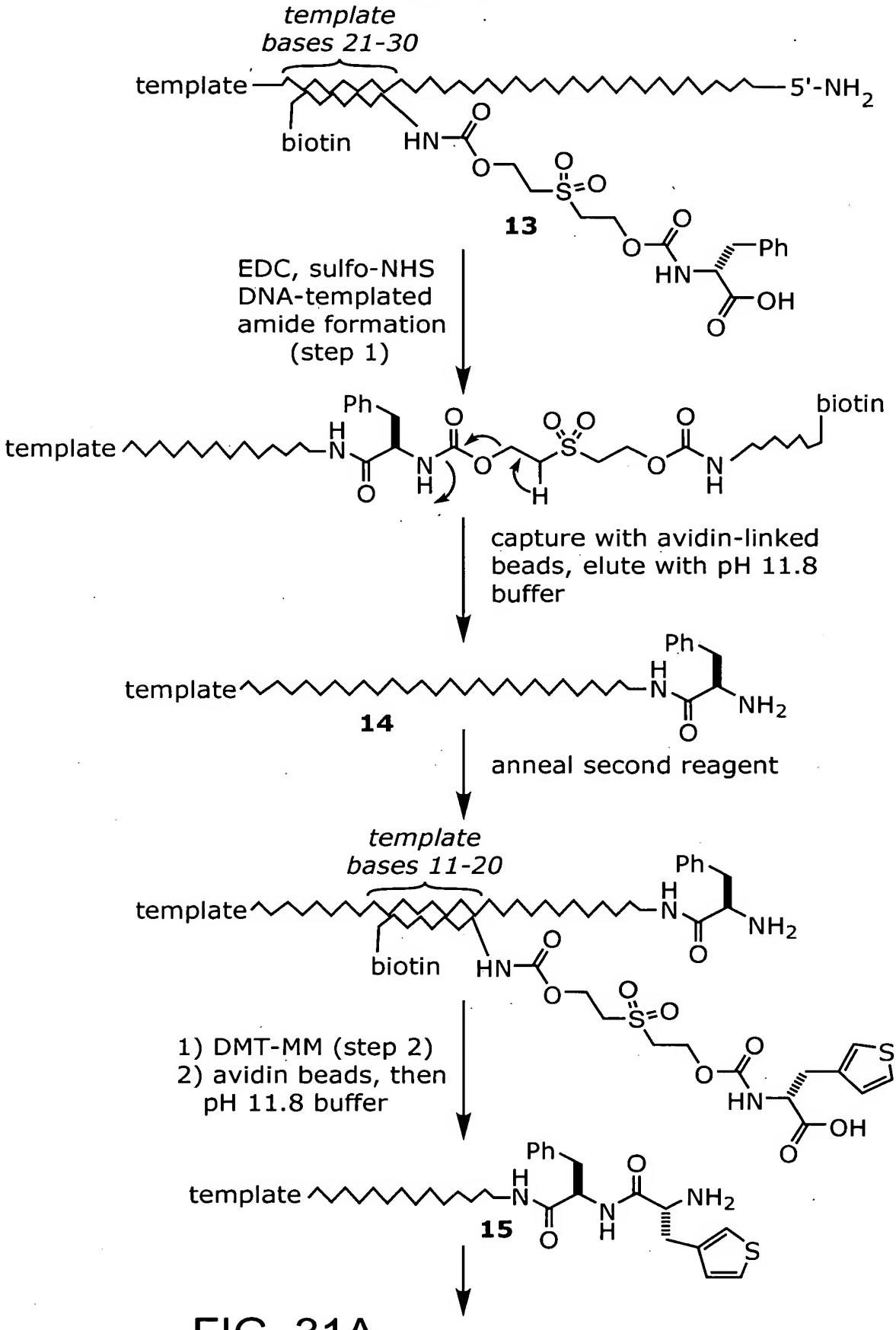
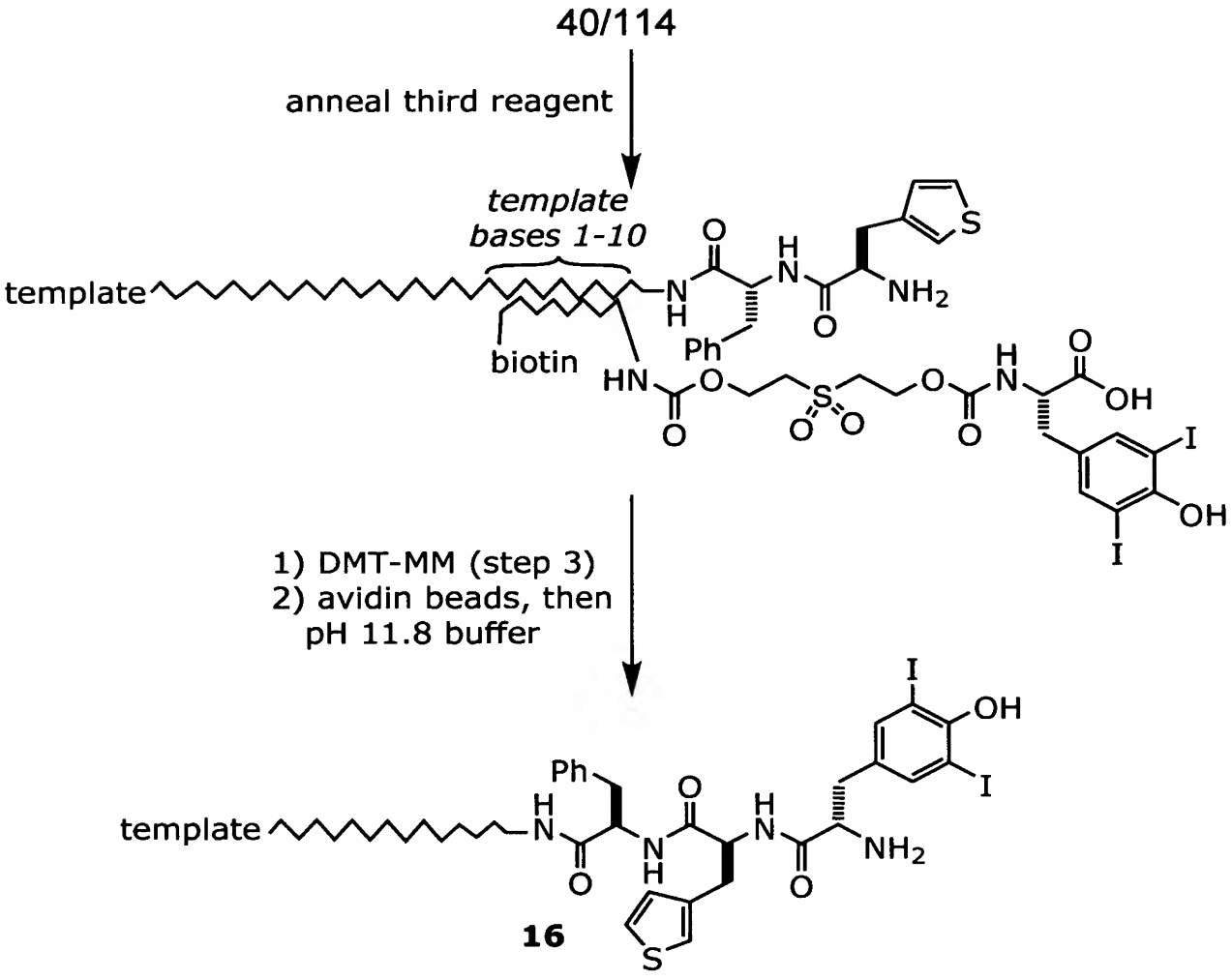

template
bases 21-30
template
5'-NH2
biotin
HN
13
EDC, sulfo-NHS
DNA-templated
amide formation
(step 1)
Ph
biotin
template
capture with avidin-linked
beads, elute with pH 11.8
buffer
template
14
NH2
anneal second reagent
template
bases 11-20
template
biotin
HN
1) DMT-MM (step 2)
2) avidin beads, then
pH 11.8 buffer
OH
template
15
NH2

FIG. 31A


anneal third reagent
template
bases 1-10
template
biotin
1) DMT-MM (step 3)
2) avidin beads, then
pH 11.8 buffer
template
16

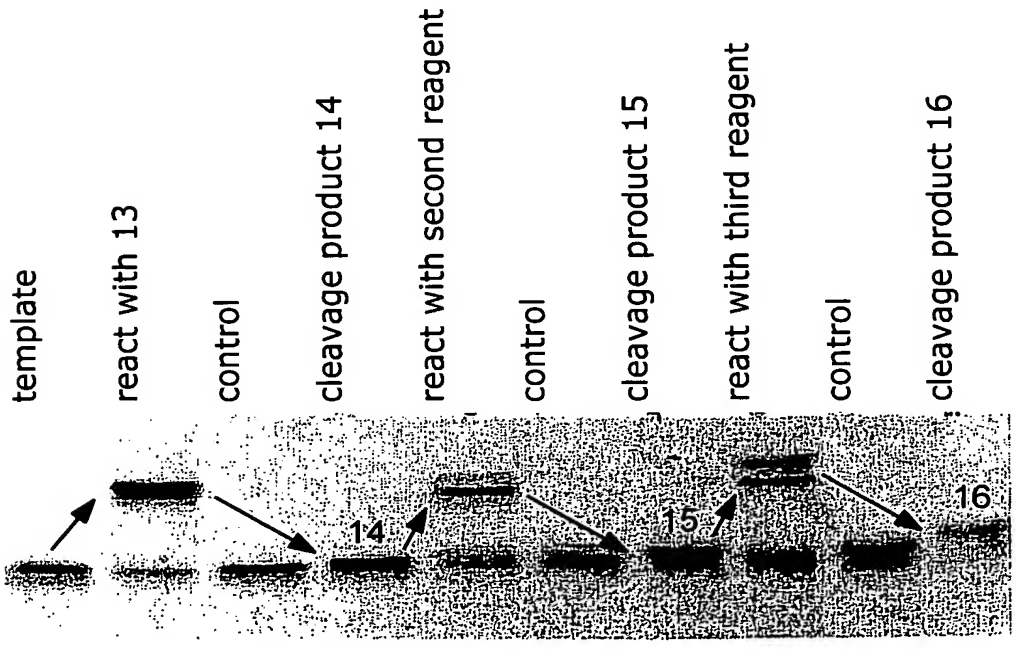

template
react with 13
control
cleavage product 14
react with second reagent
control
cleavage product 15
react with third reagent
control
cleavage product 16
14
15
16

FIG. 31B

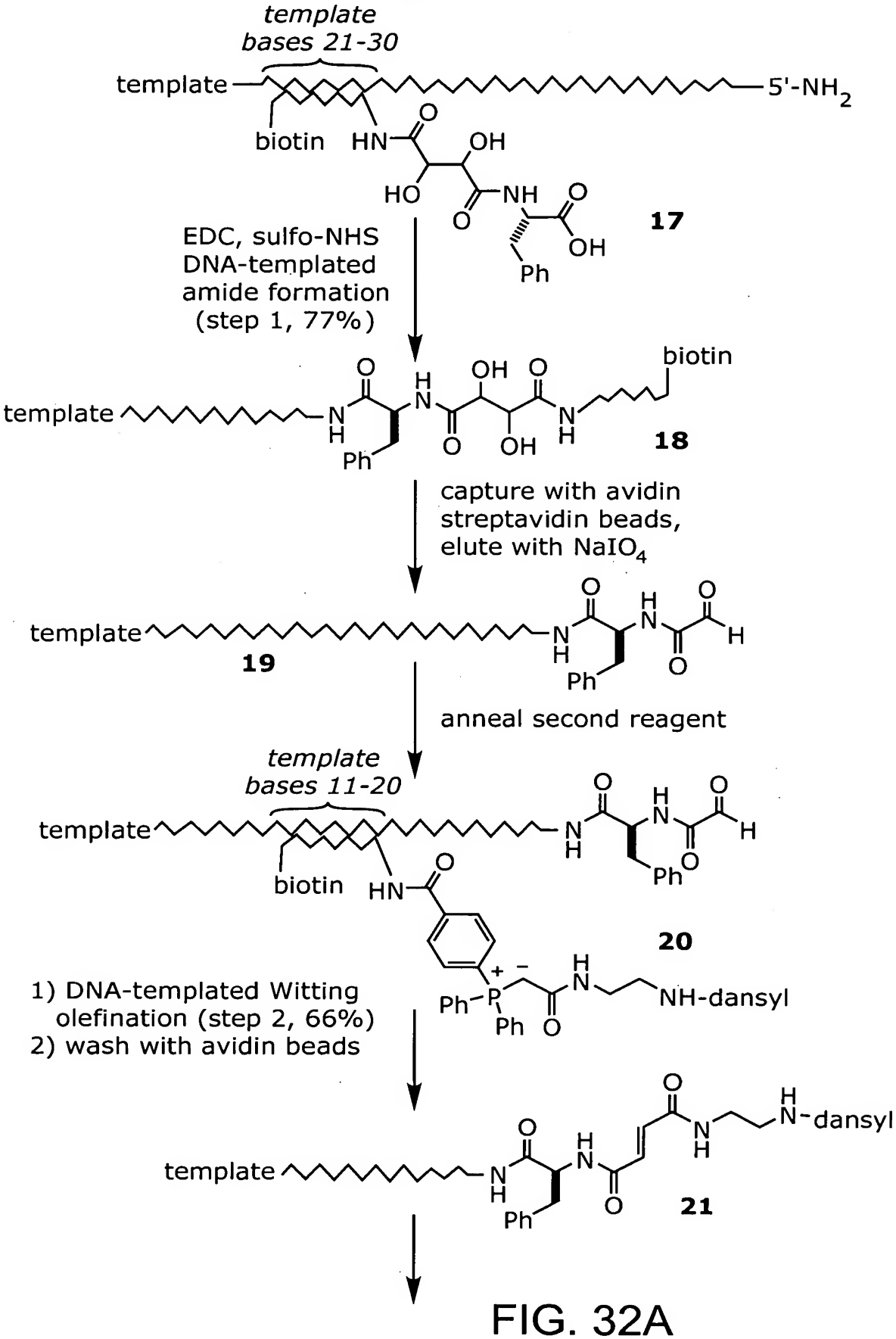

template
bases 21-30
template
5'-NH2
biotin
HN
OH
HO
NH
OH
Ph
17
EDC, sulfo-NHS
DNA-templated
amide formation
(step 1, 77%)
biotin
template
Ph
18
capture with avidin
streptavidin beads,
elute with NaIO4
template
19
anneal second reagent
template
bases 11-20
template
biotin
HN
20
1) DNA-templated Witting
olefination (step 2, 66%)
2) wash with avidin beads
NH-dansyl
N-dansyl
template
21

FIG. 32A

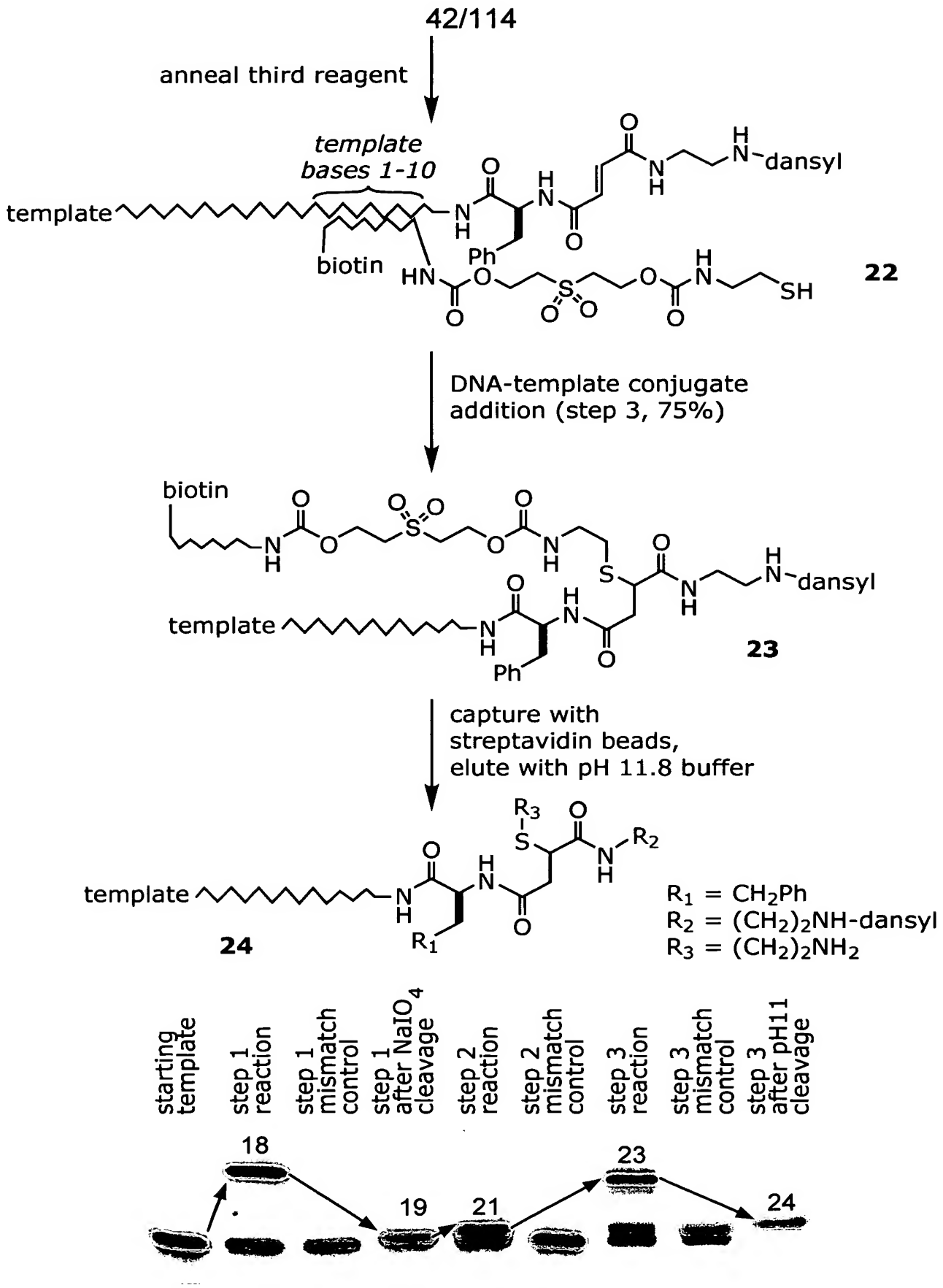

anneal third reagent
template
template bases 1-10
biotin
dansyl
22
DNA-template conjugate addition (step 3, 75%)
biotin
template
dansyl
23
capture with streptavidin beads, elute with pH 11.8 buffer
template
24
$R_1 = CH_2Ph$
$R_2 = (CH_2)_2NH$-dansyl
$R_3 = (CH_2)_2NH_2$
starting template
step 1 reaction
step 1 mismatch control
step 1 after $NaIO_4$ cleavage
step 2 reaction
step 2 mismatch control
step 3 reaction
step 3 mismatch control
step 3 after pH11 cleavage
18
19
21
23
24

FIG. 32B

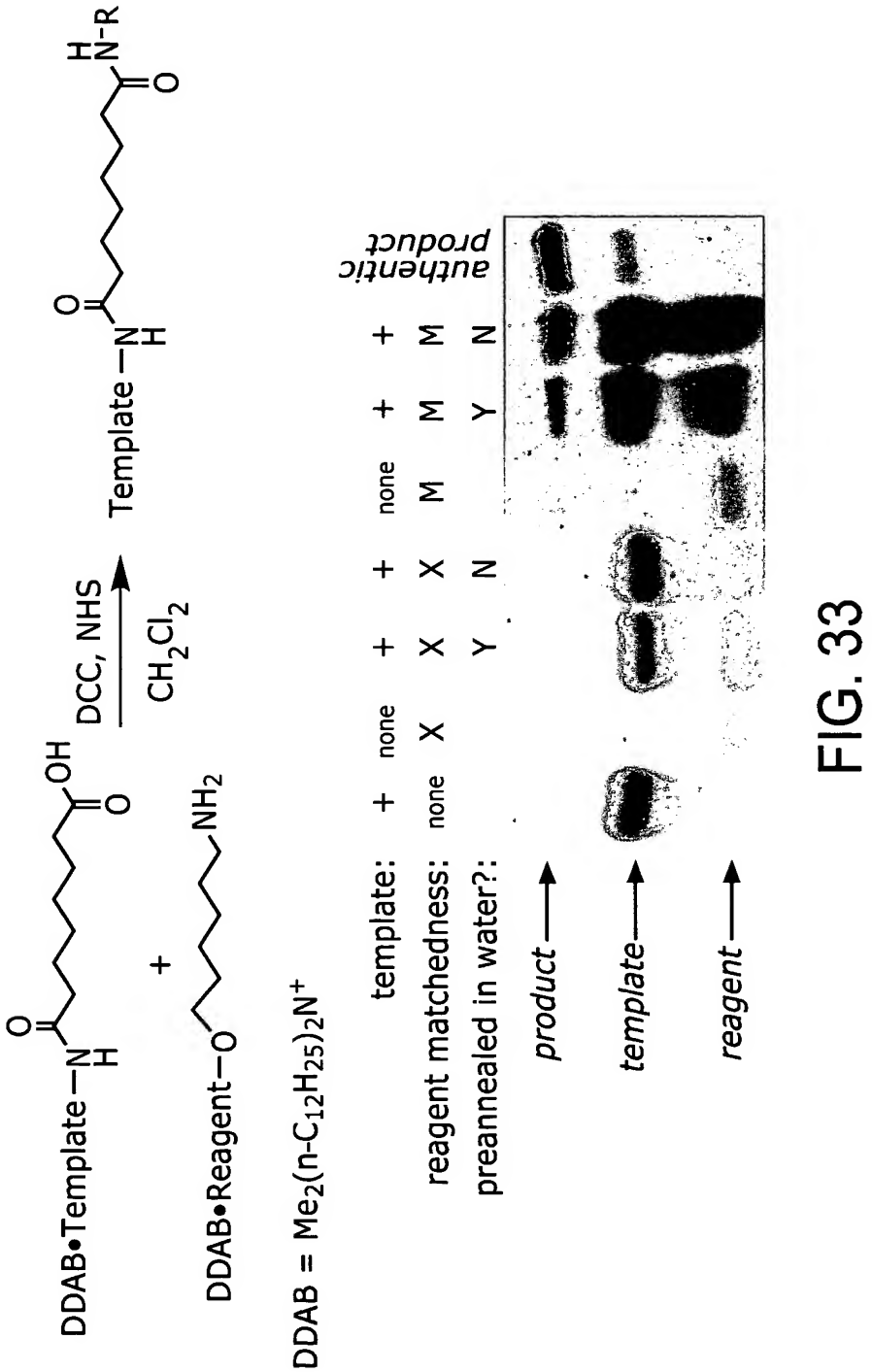

DDAB•Template—N(H)
O
OH
O
+
DDAB•Reagent—O
NH2
DCC, NHS
CH2Cl2
Template—N(H)
H
N-R
O
DDAB = Me2(n-C12H25)2N+
template: + none + + + none + + +
reagent matchedness: none X X X M M M N
preannealed in water?: Y Y N Y
product
template
reagent
authentic product

FIG. 33

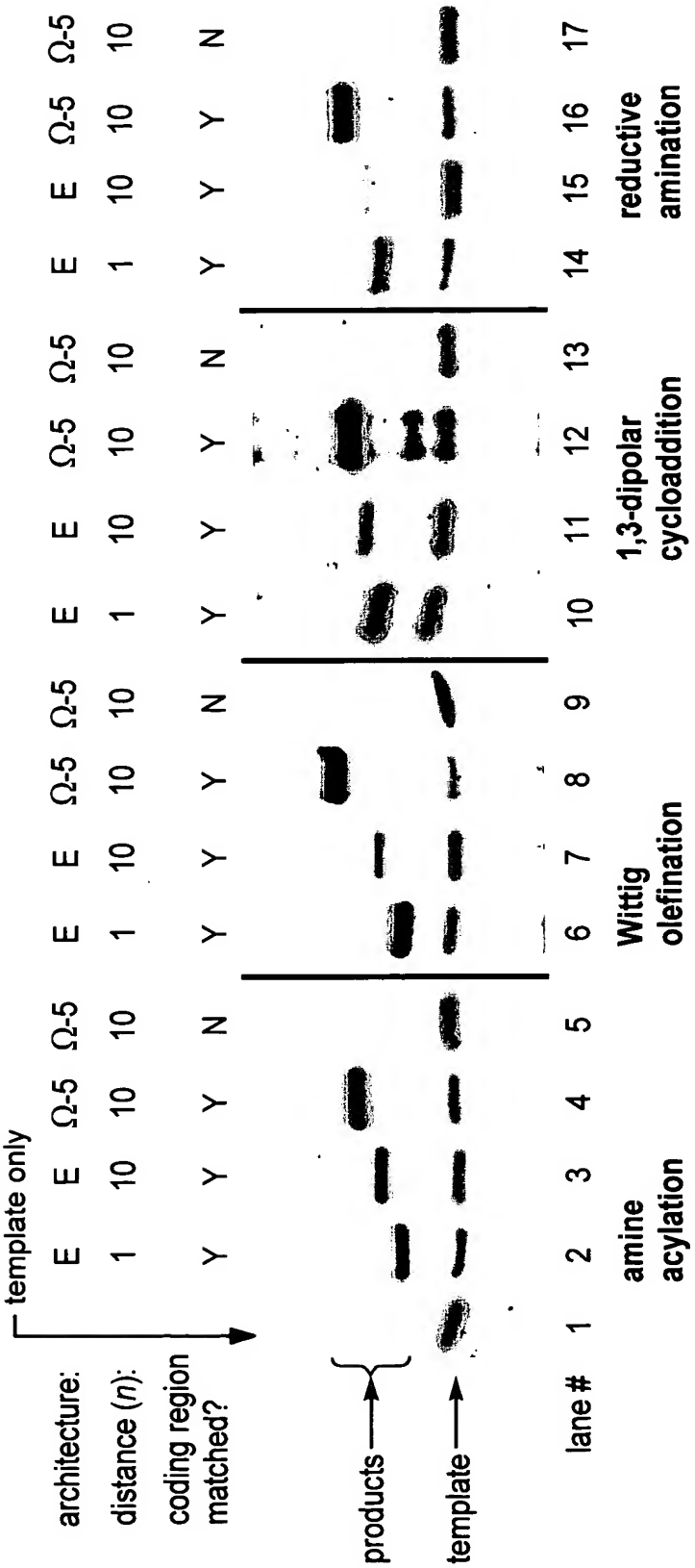

template only
architecture:
distance (n):
coding region matched?
E 1 Y
E 10 Y
Ω-5 10 Y
Ω-5 10 N
E 1 Y
E 10 Y
Ω-5 10 Y
Ω-5 10 N
E 1 Y
E 10 Y
Ω-5 10 Y
Ω-5 10 N
E 1 Y
E 10 Y
Ω-5 10 Y
Ω-5 10 N
products
template
lane # 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
amine acylation
Wittig olefination
1,3-dipolar cycloaddition
reductive amination

FIG. 34

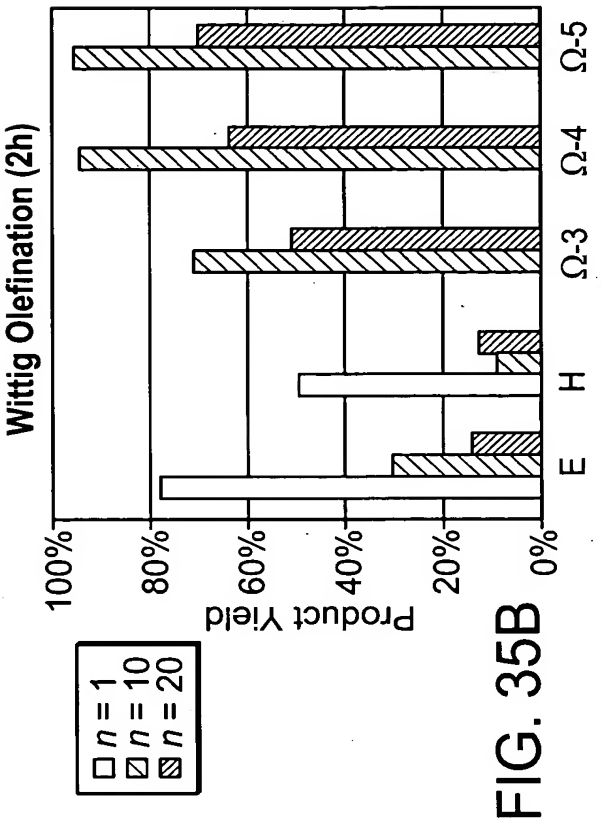


FIG. 35B

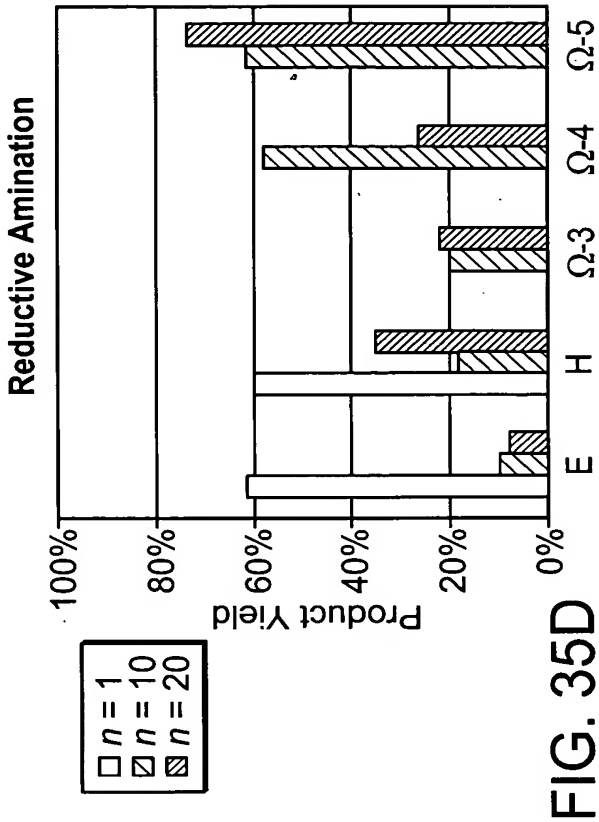


FIG. 35D

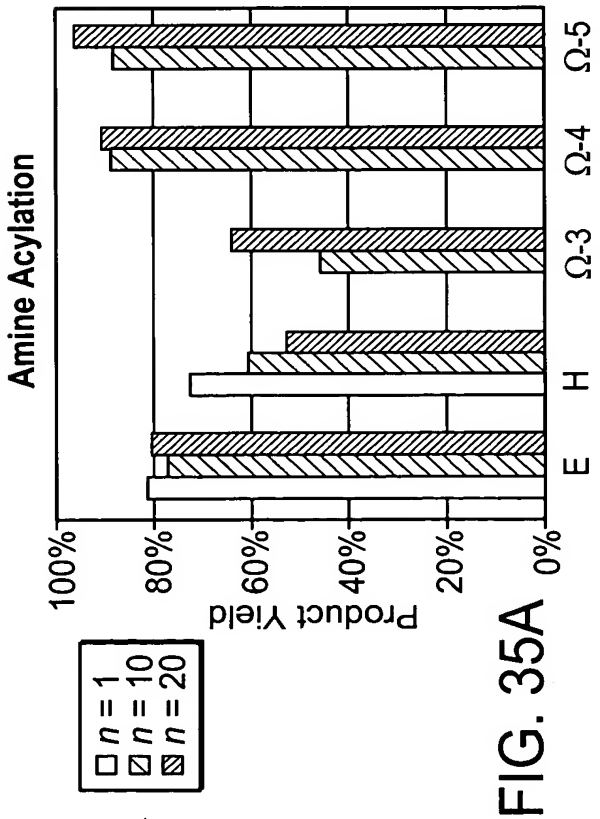


FIG. 35A

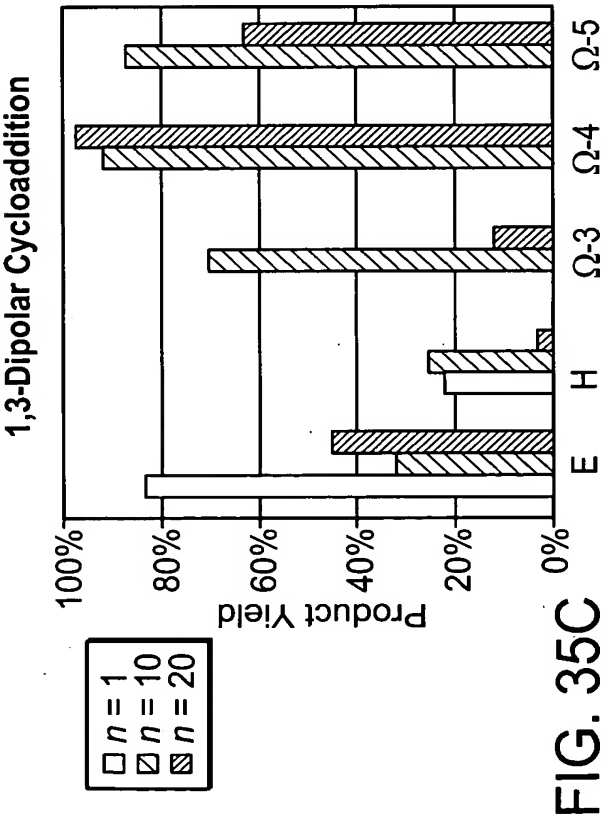


FIG. 35C

Architecture	Buffer	T_m (°C)
E (n = 10)	PBS	45
Ω (n = 10)	PBS	46
E (n = 10)	HSP	55
Ω (n = 10)	HSP	54
E (n = 20)	PBS	40
Ω (n = 20)	PBS	39

FIG. 36

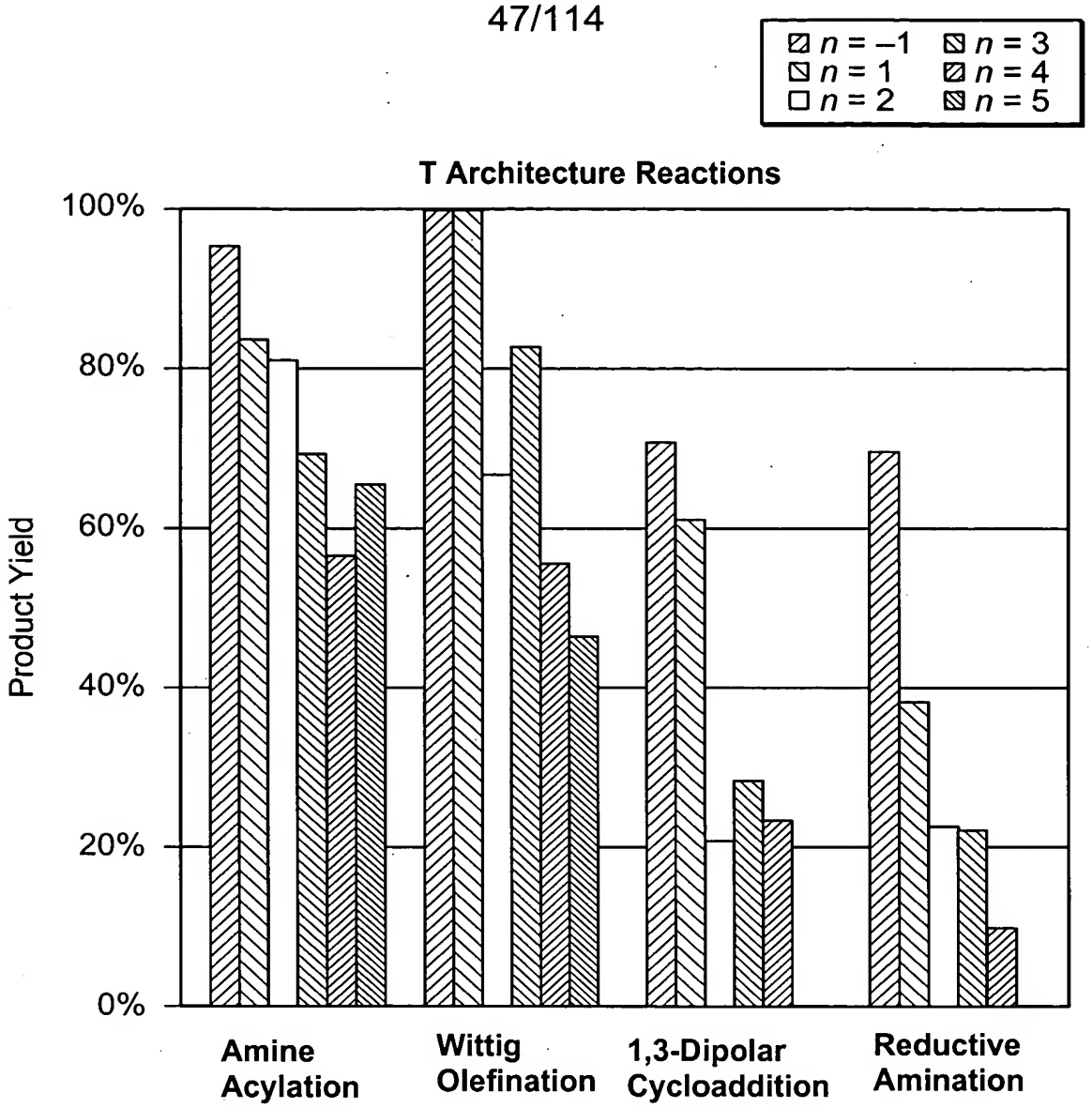


FIG. 37

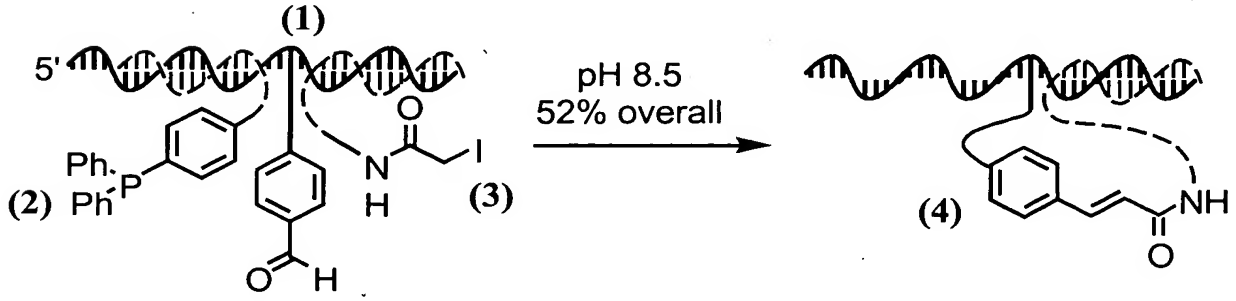


FIG. 38A

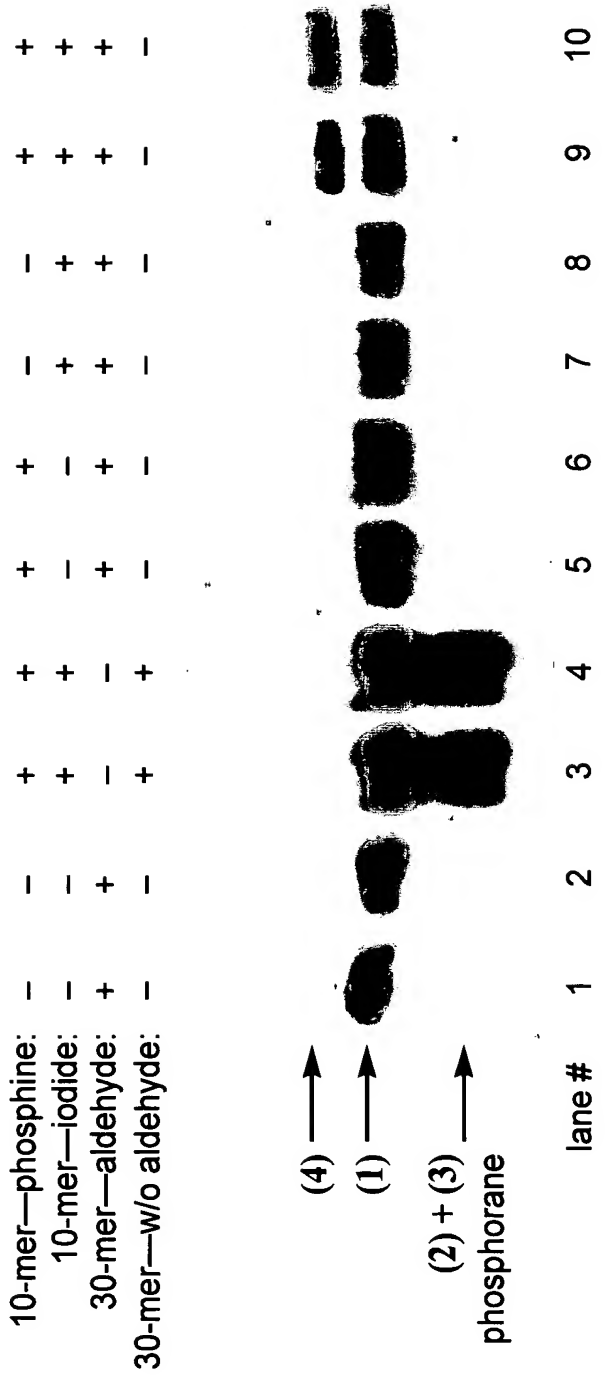
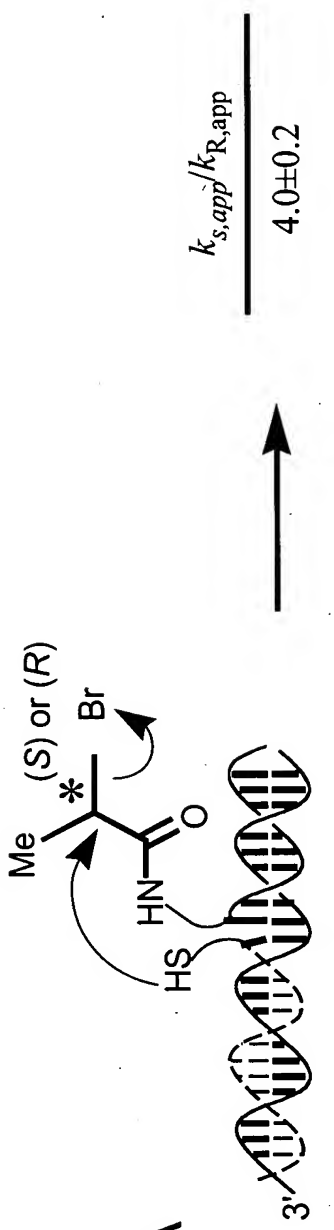


FIG. 38B

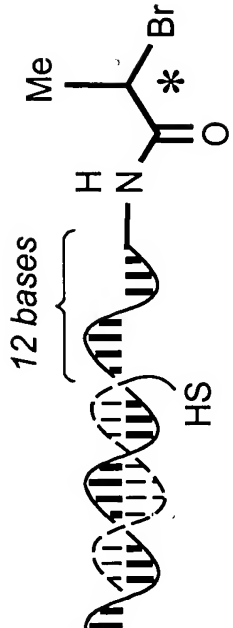
FIG. 38C



$k_{s,app}/k_{R,app}$

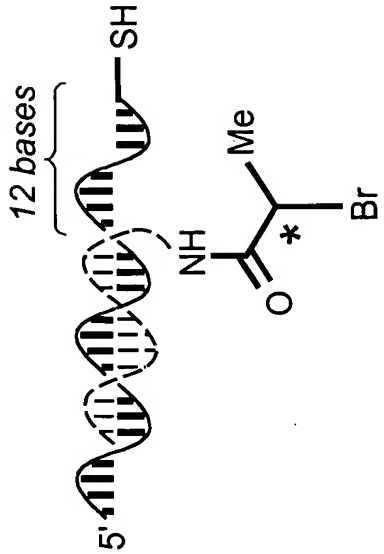
4.0±0.2

FIG. 39A



5.0±0.7

FIG. 39B



4.6±1.1

FIG. 39C

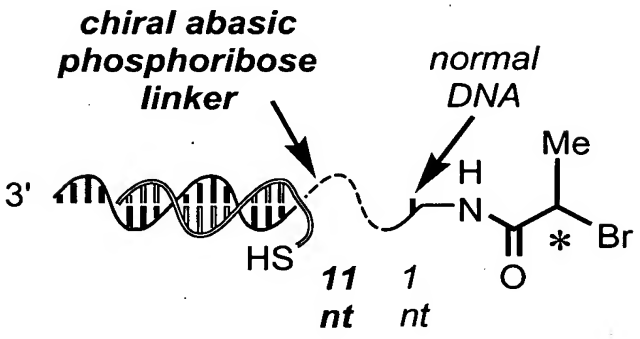


FIG. 40A

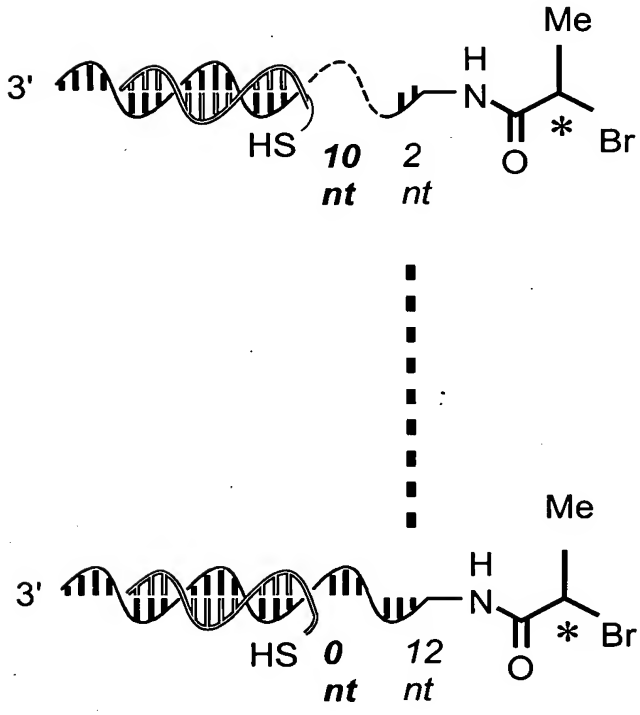


FIG. 40B

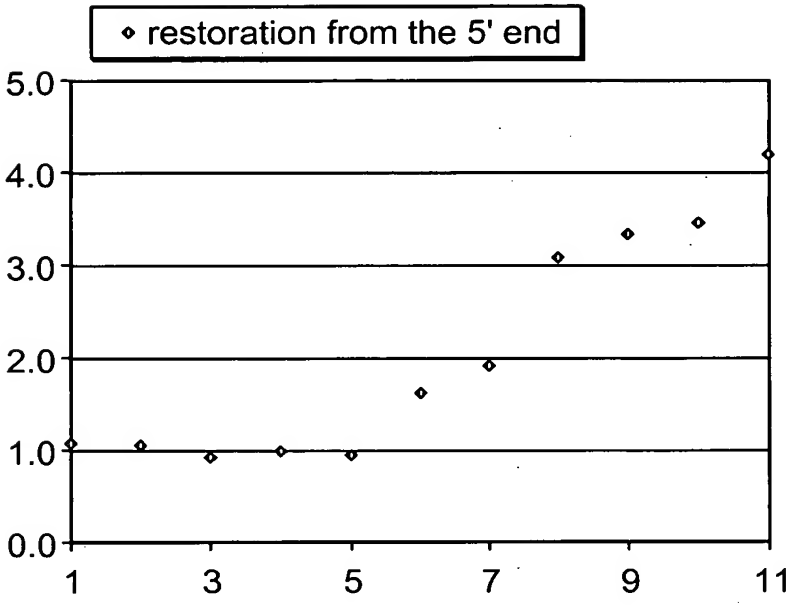


FIG. 40C

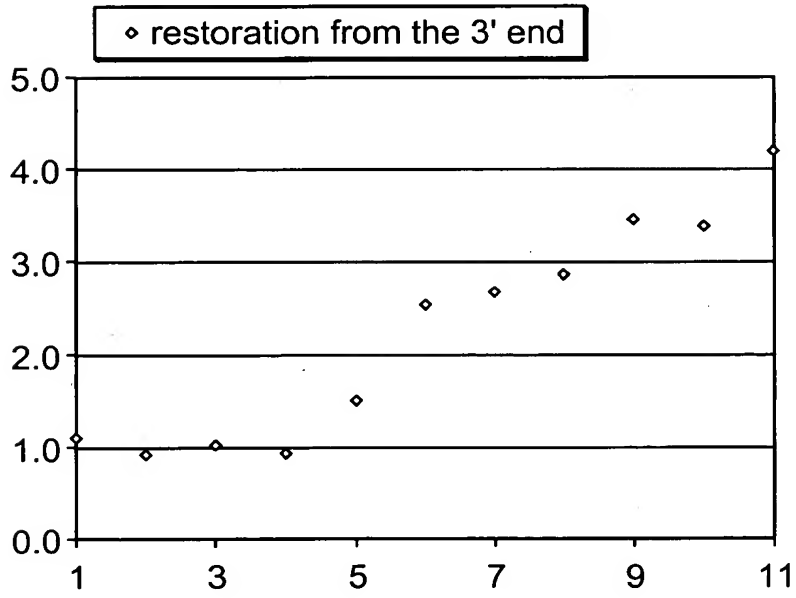


FIG. 40D

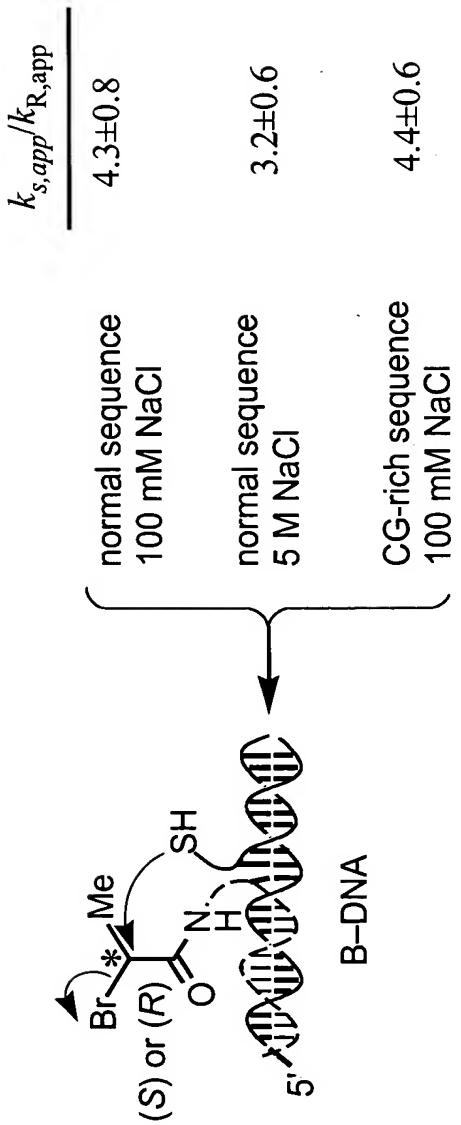


FIG. 41A

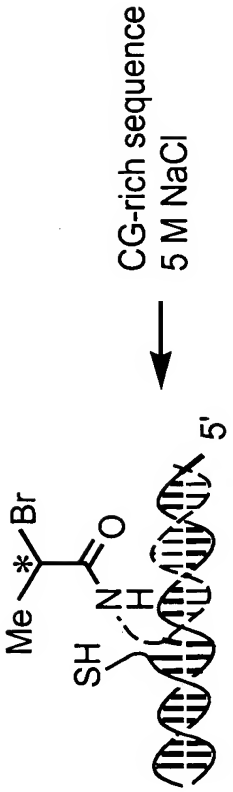


FIG. 41B

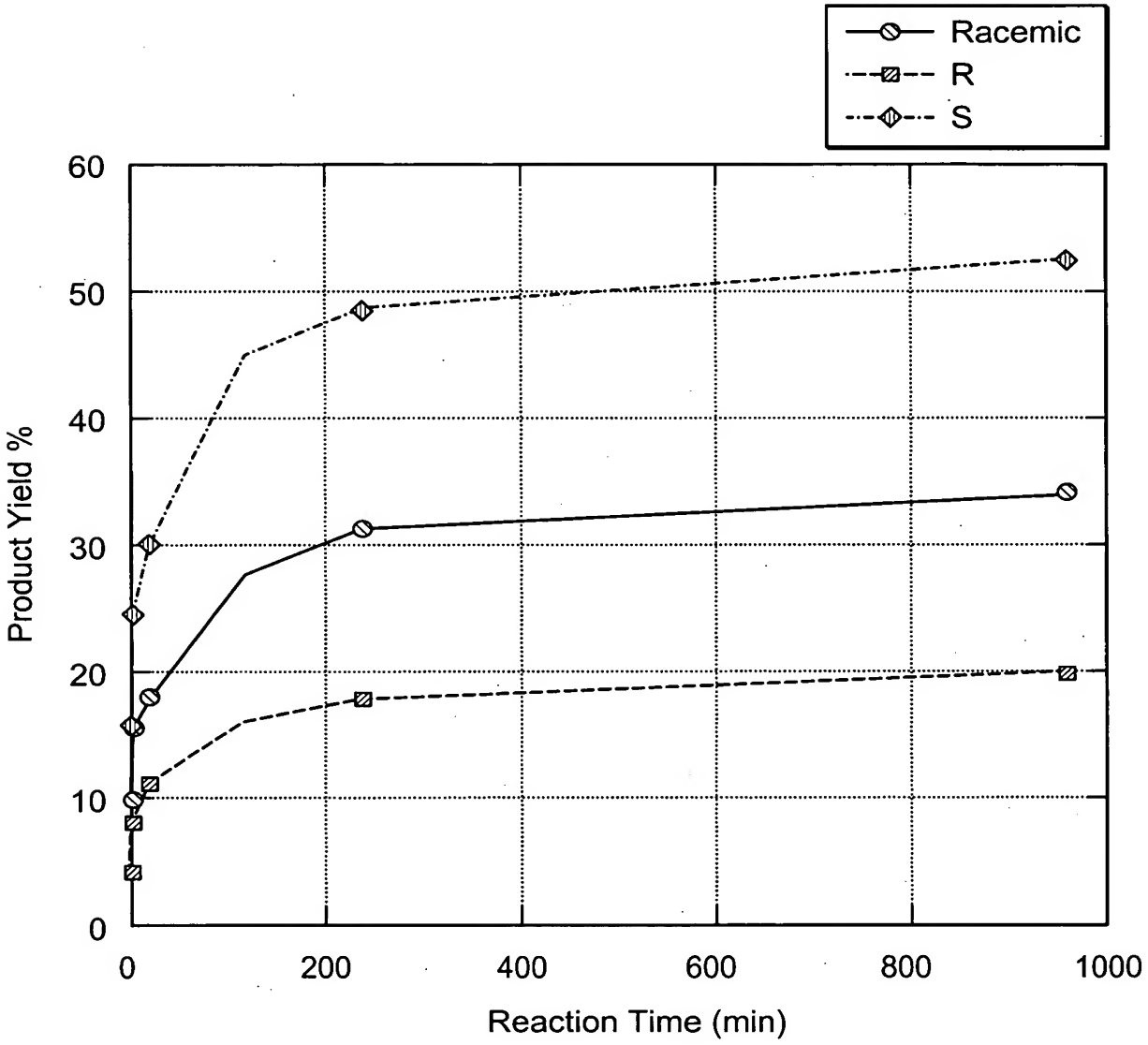

Racemic
R
S
Product Yield %
0
10
20
30
40
50
60
0
200
400
600
800
1000
Reaction Time (min)

FIG. 42A

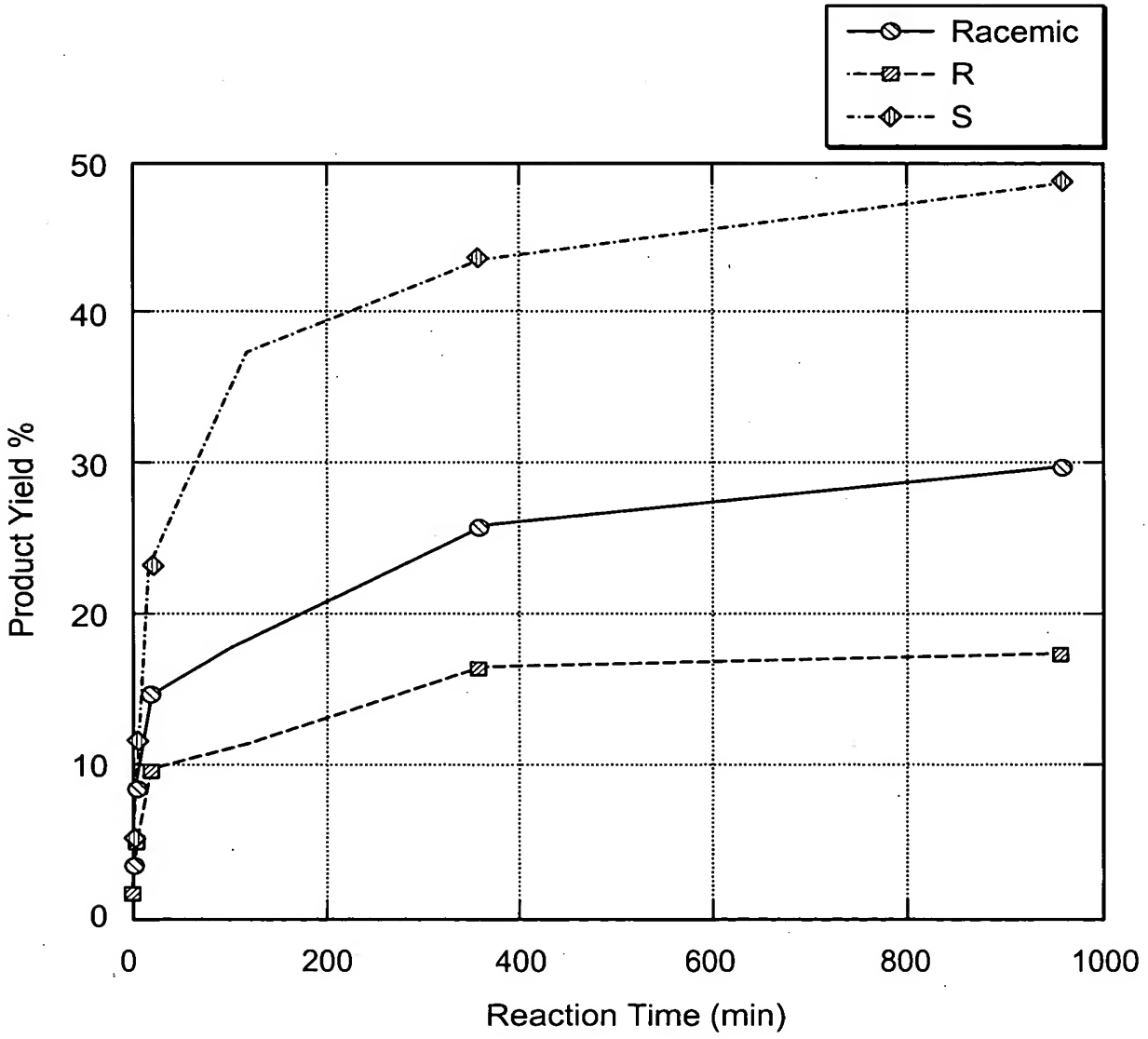

Racemic
R
S
Product Yield %
50
40
30
20
10
0
0
200
400
600
800
1000
Reaction Time (min)

FIG. 42B

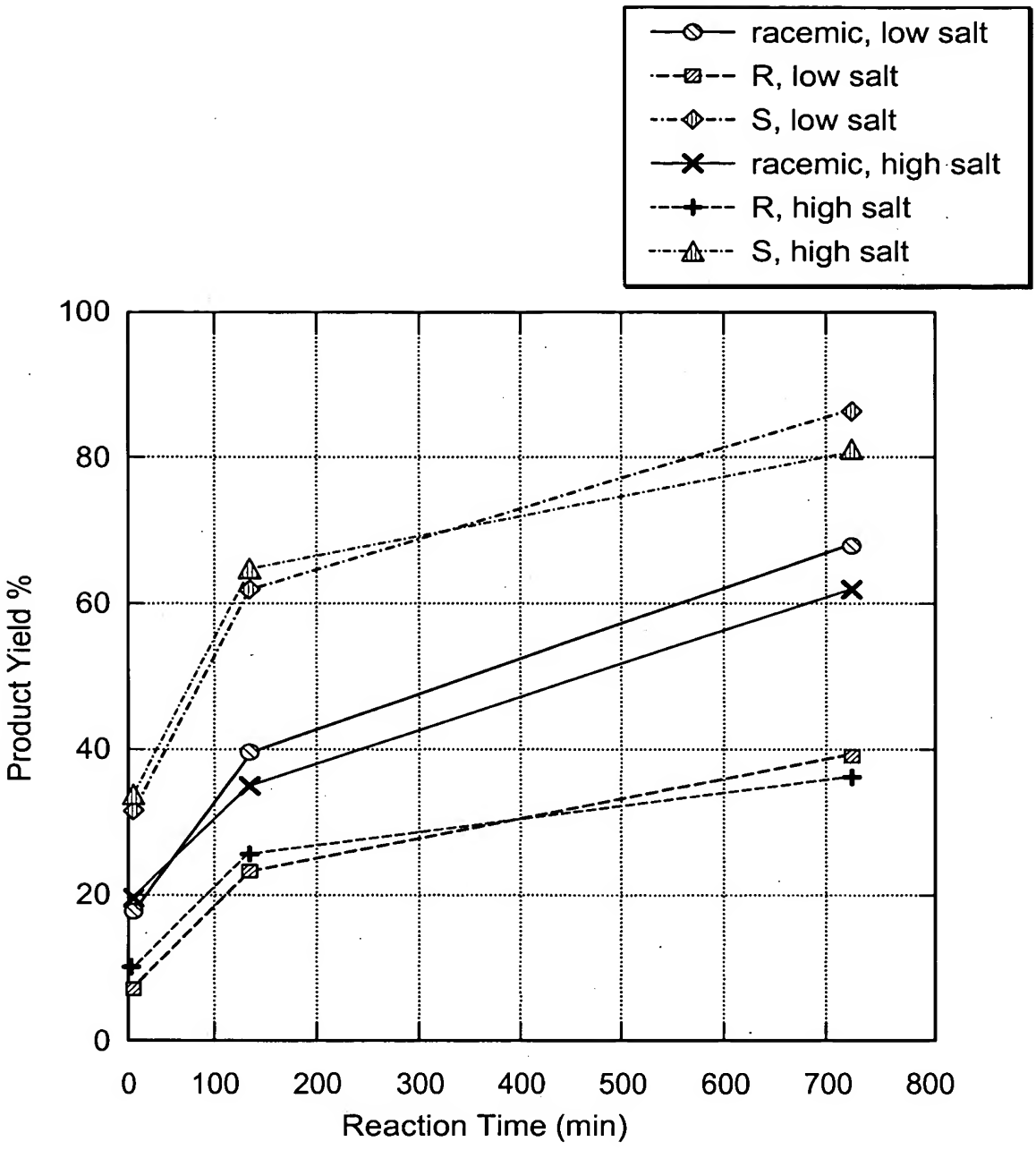

racemic, low salt
R, low salt
S, low salt
racemic, high salt
R, high salt
S, high salt
Product Yield %
100
80
60
40
20
0
0
100
200
300
400
500
600
700
800
Reaction Time (min)

FIG. 42C

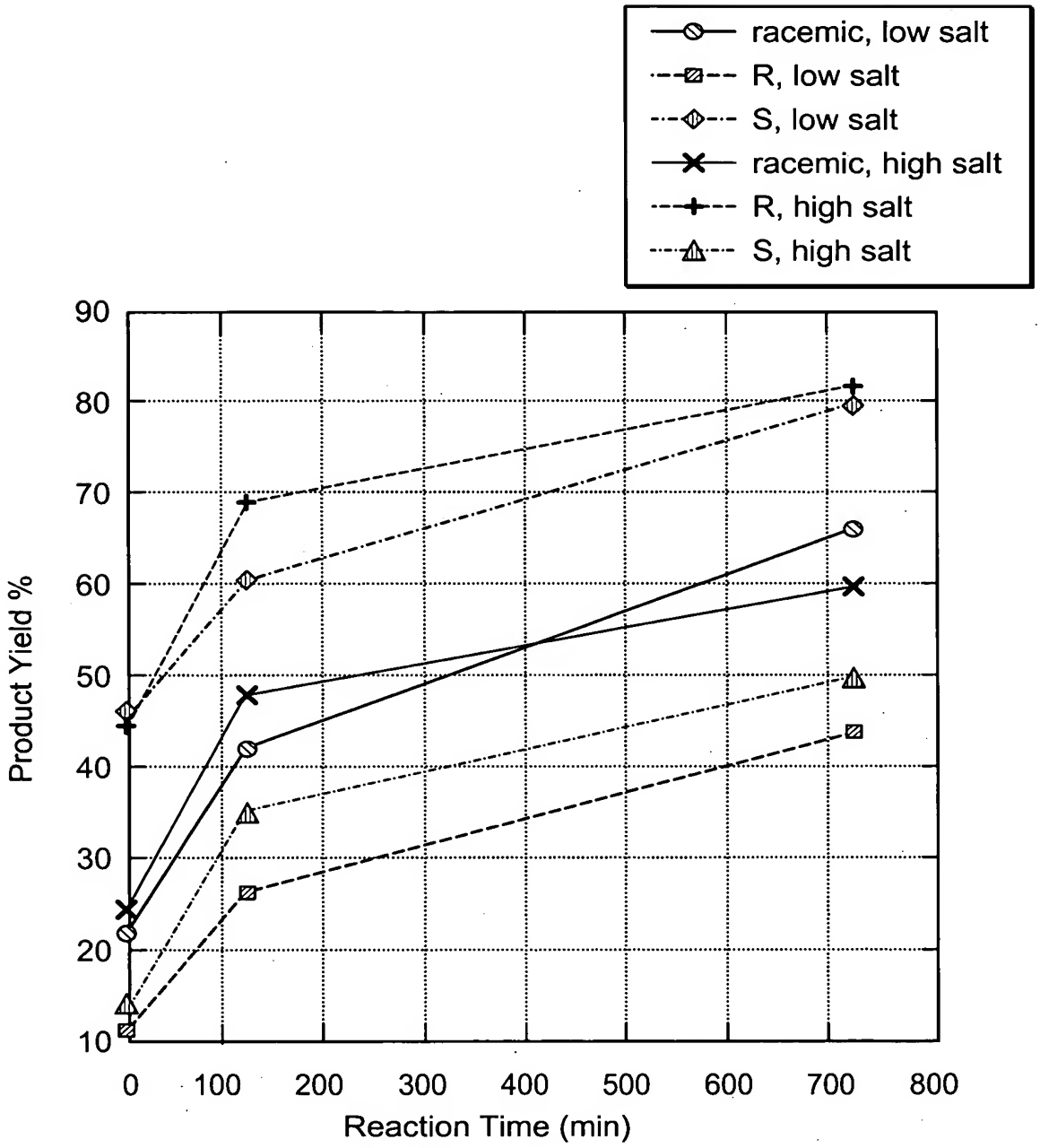
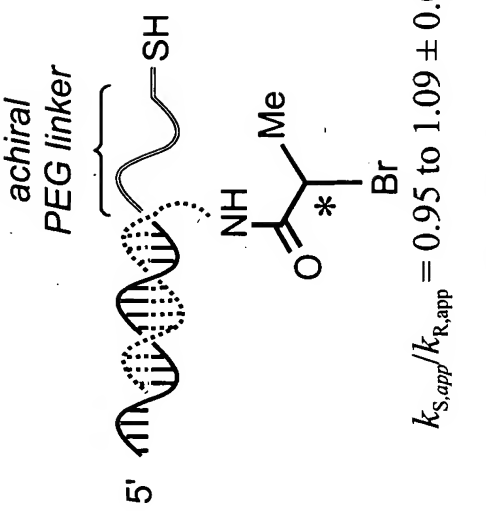
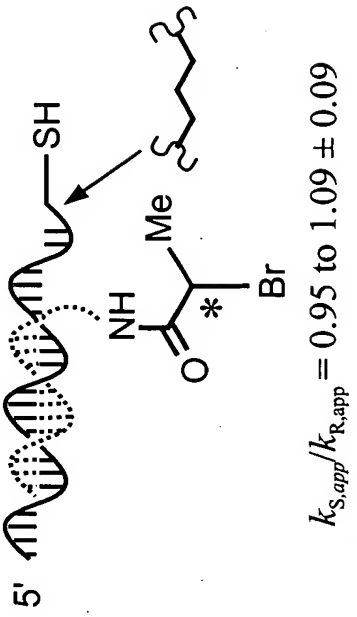

racemic, low salt
R, low salt
S, low salt
racemic, high salt
R, high salt
S, high salt
Product Yield %
90
80
70
60
50
40
30
20
10
0
100
200
300
400
500
600
700
800
Reaction Time (min)

FIG. 42D



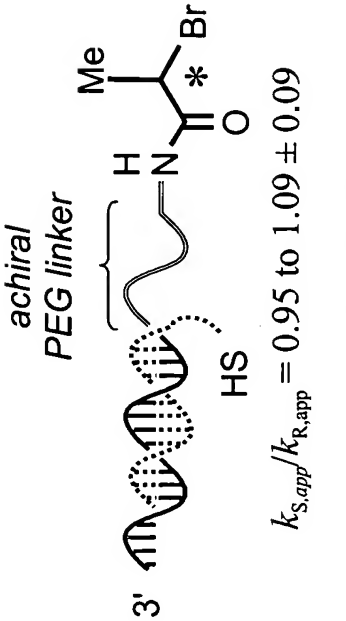
$k_{S,app}/k_{R,app} = 0.95$ to 1.09 ± 0.09

FIG. 43B



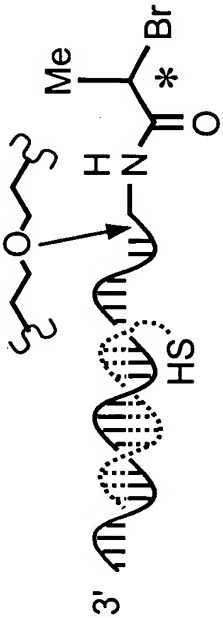
$k_{S,app}/k_{R,app} = 0.95$ to 1.09 ± 0.09

FIG. 43D



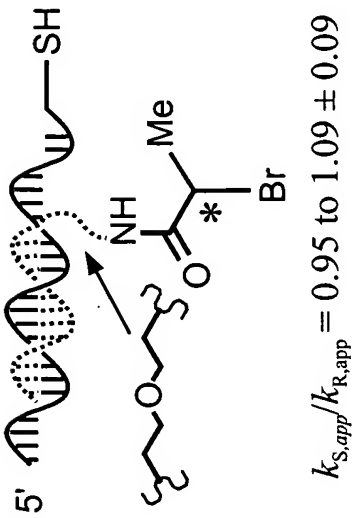
$k_{S,app}/k_{R,app} = 0.95$ to 1.09 ± 0.09

FIG. 43A



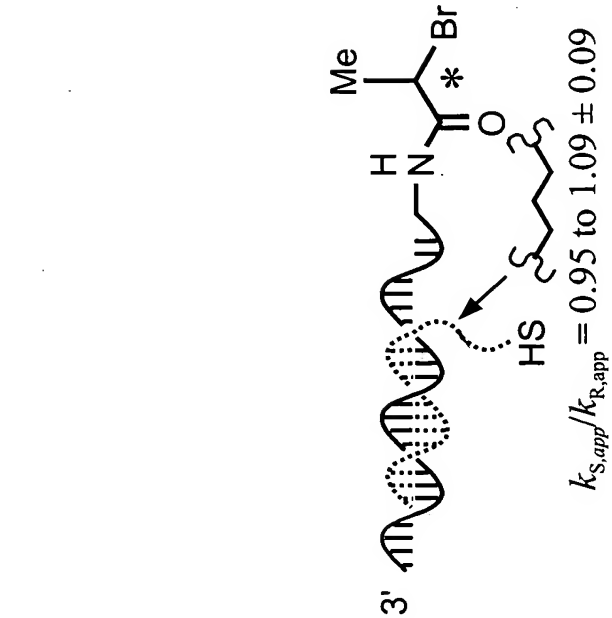
$k_{S,app}/k_{R,app} = 0.95$ to 1.09 ± 0.09

FIG. 43C



$k_{S,app}/k_{R,app} = 0.95$ to 1.09 ± 0.09

FIG. 43F



$k_{S,app}/k_{R,app} = 0.95$ to 1.09 ± 0.09

FIG. 43E

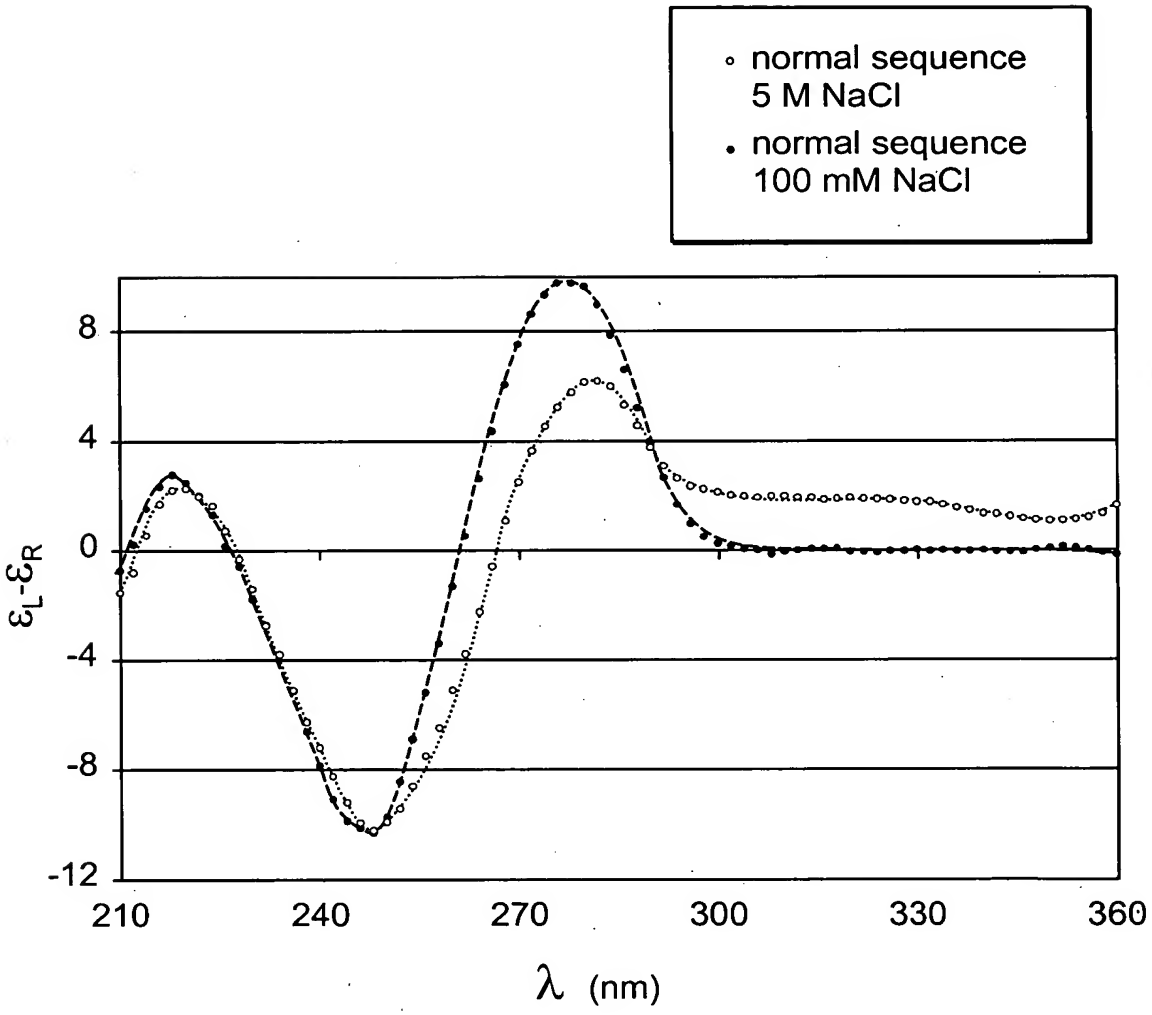

normal sequence
5 M NaCl
normal sequence
100 mM NaCl
8
4
0
-4
-8
-12
ε_L-ε_R
210
240
270
300
330
360
λ (nm)

FIG. 44A

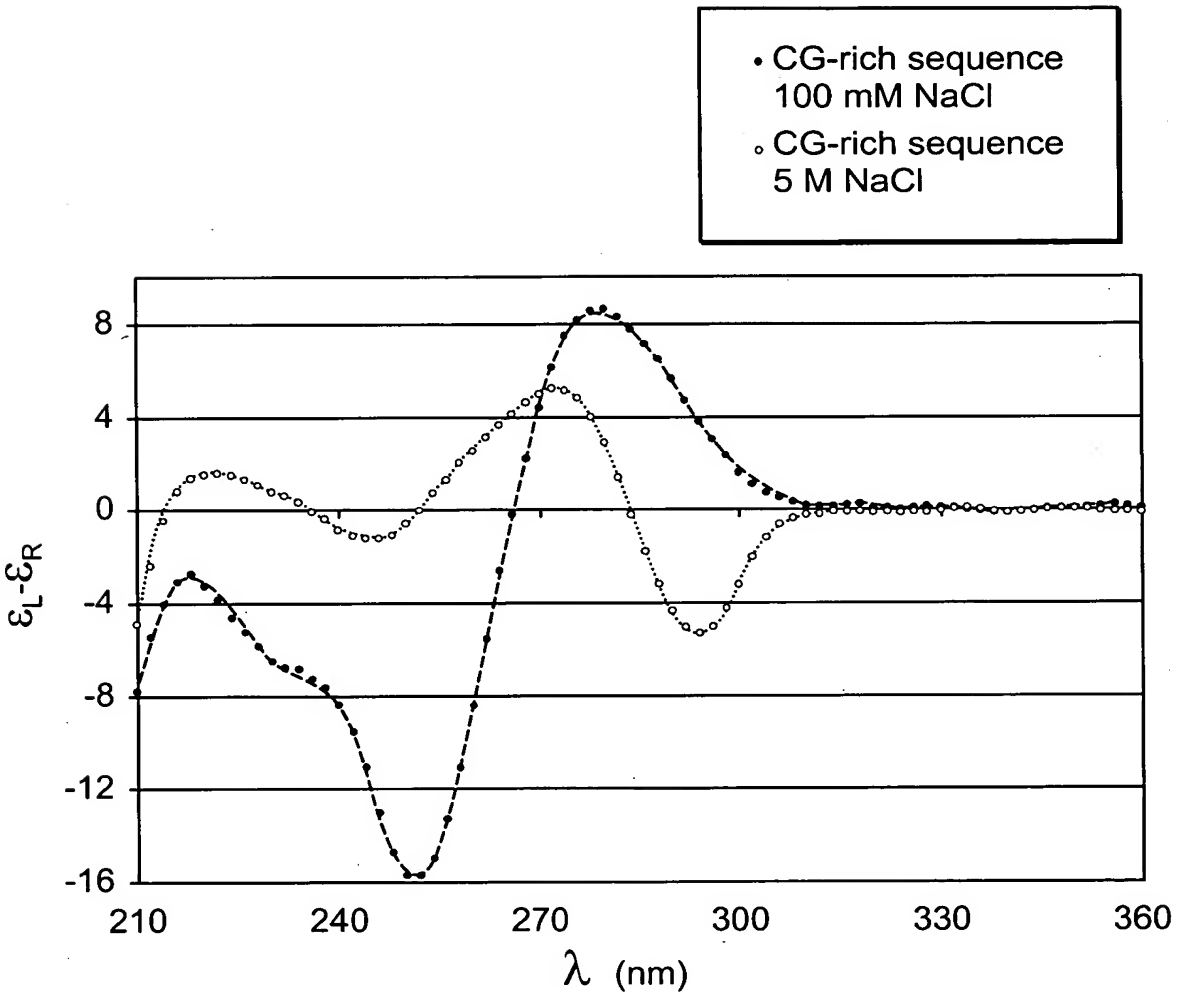

• CG-rich sequence 100 mM NaCl
◦ CG-rich sequence 5 M NaCl
8
4
0
-4
-8
-12
-16
ε_L-ε_R
210
240
270
300
330
360
λ (nm)

FIG. 44B

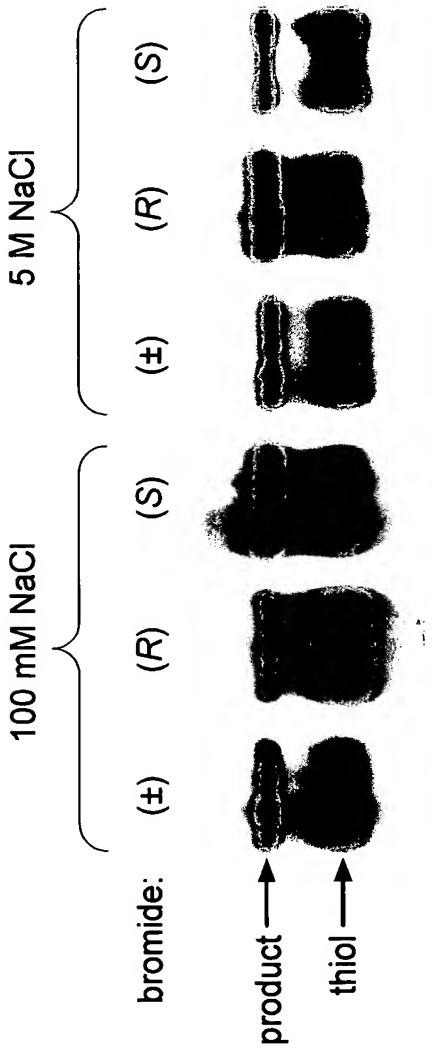


FIG. 45

FIG. 46A	FIG. 46B	FIG. 46C

FIG. 46

Title: Evolving New Molecular Function
Inventor: Liu *et al.*
Serial No.: 10/643,752
Atty Docket No.: LS5-001 (9792/5)
Atty/Agent: Brian A. Fairchild
Express Mail Label No.: EL932952135US
Sheet 62 of 114

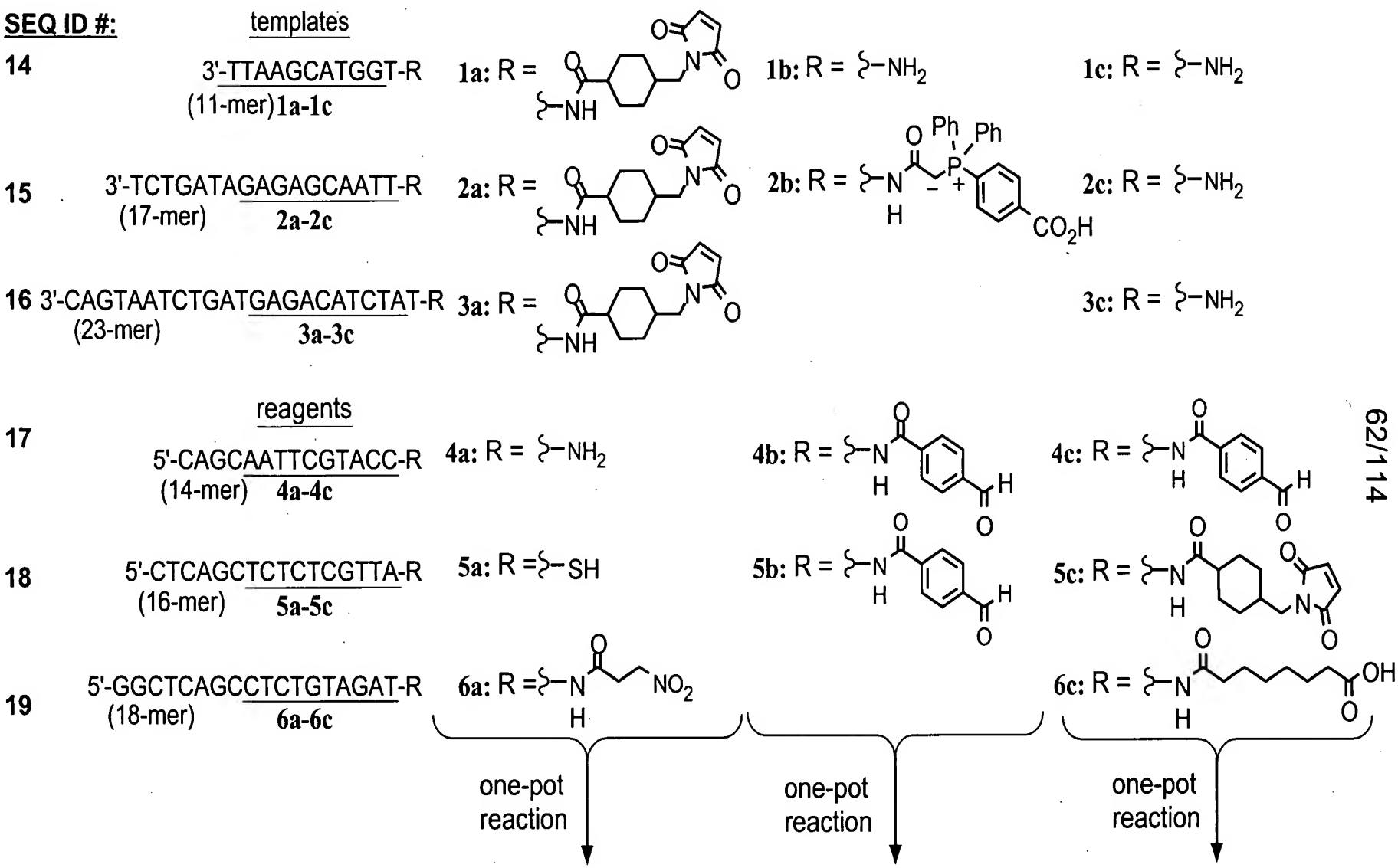


FIG. 46A

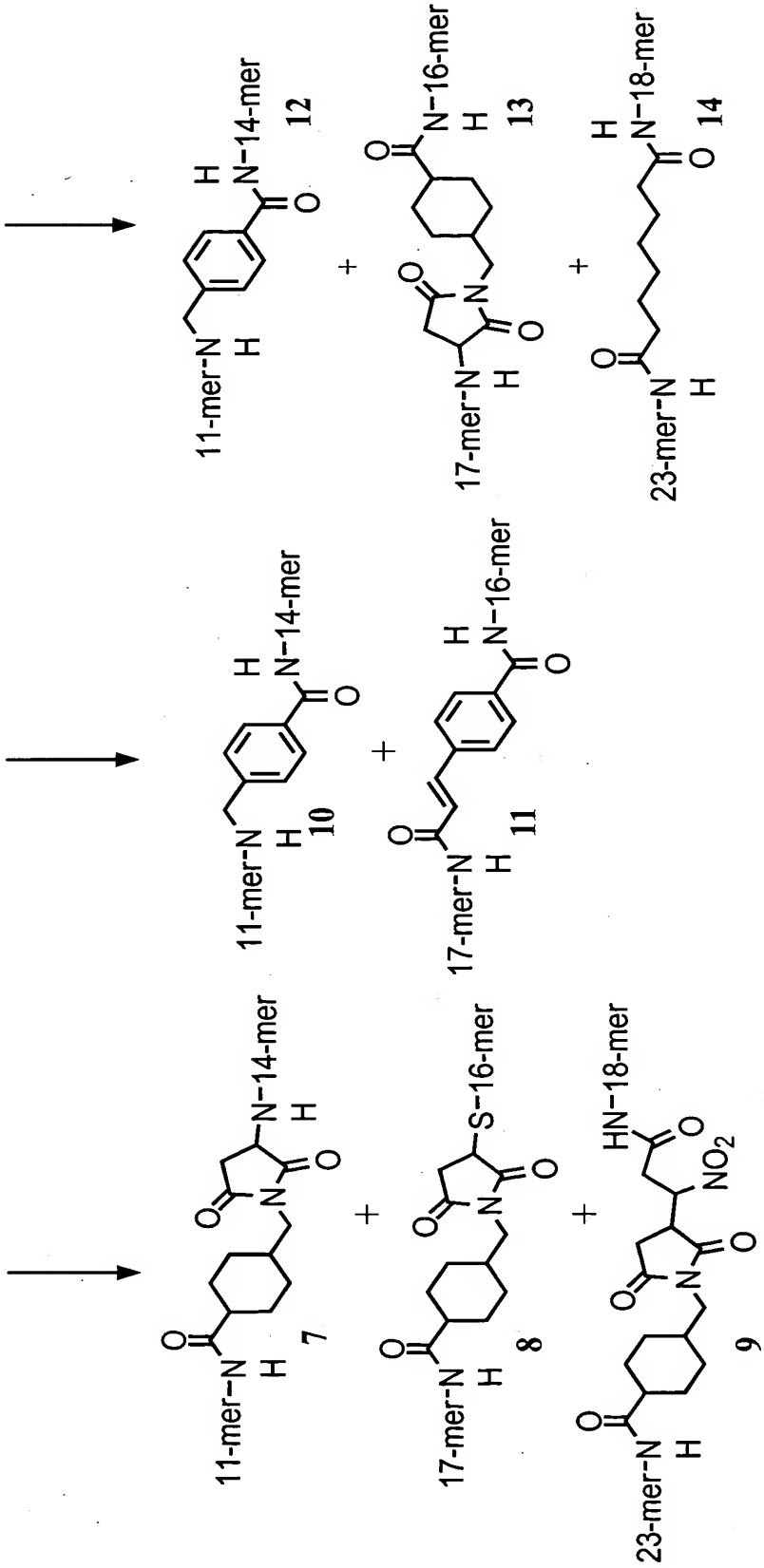

11-mer-N
H
N-14-mer
O
12
+
17-mer-N
H
N-16-mer
O
13
+
23-mer-N
H
N-18-mer
O
14
11-mer-N
H
N-14-mer
O
10
+
17-mer-N
H
N-16-mer
O
11
11-mer-N
H
N-14-mer
O
7
+
17-mer-N
H
S-16-mer
O
8
+
23-mer-N
H
HN-18-mer
O
NO2
9

FIG. 46B

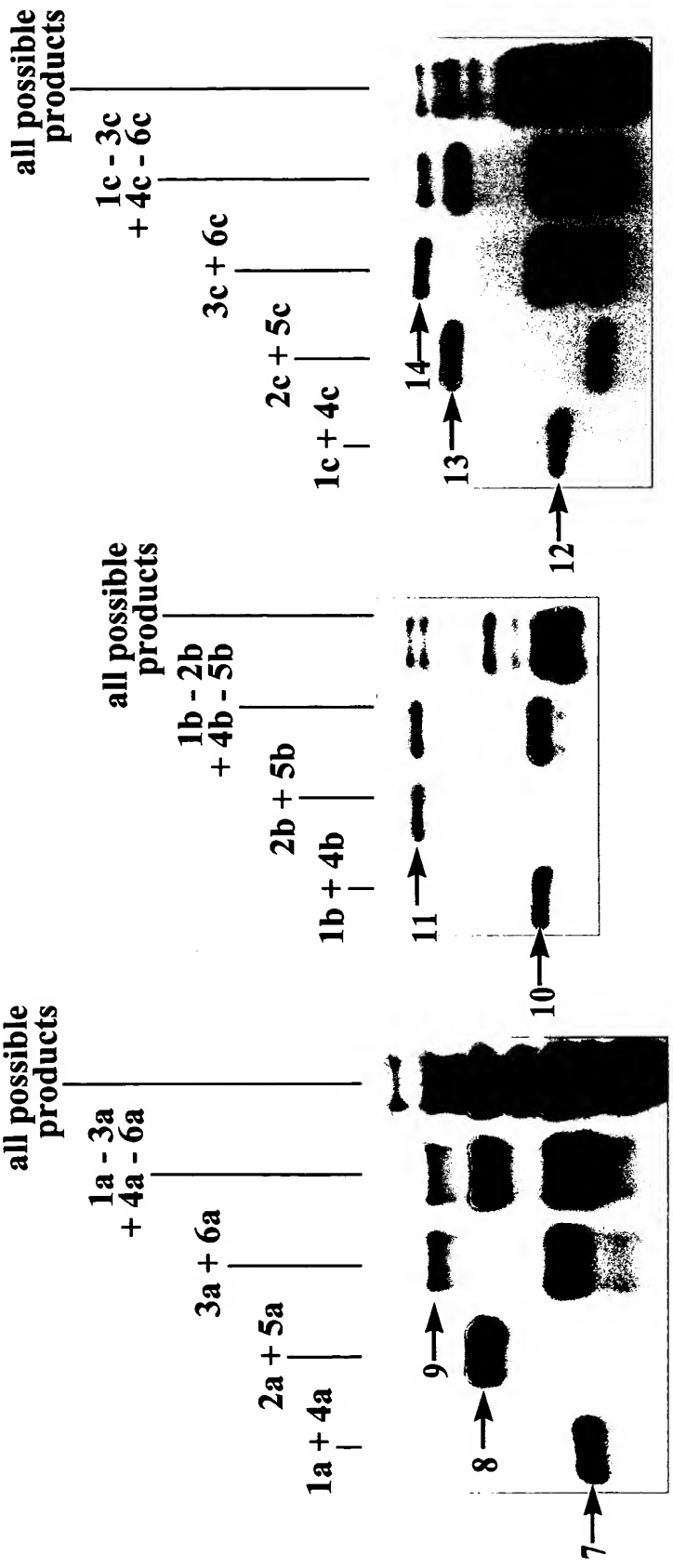

all possible products
1a - 3a + 4a - 6a
3a + 6a
2a + 5a
1a + 4a
7
8
9
all possible products
1b - 2b + 4b - 5b
2b + 5b
1b + 4b
10
11
all possible products
1c - 3c + 4c - 6c
3c + 6c
2c + 5c
1c + 4c
12
13
14

FIG. 46C

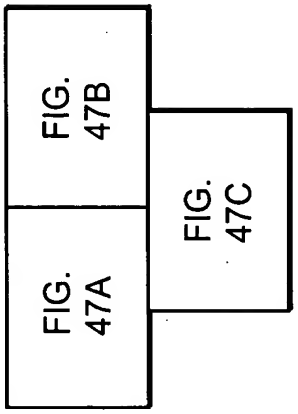


FIG. 47

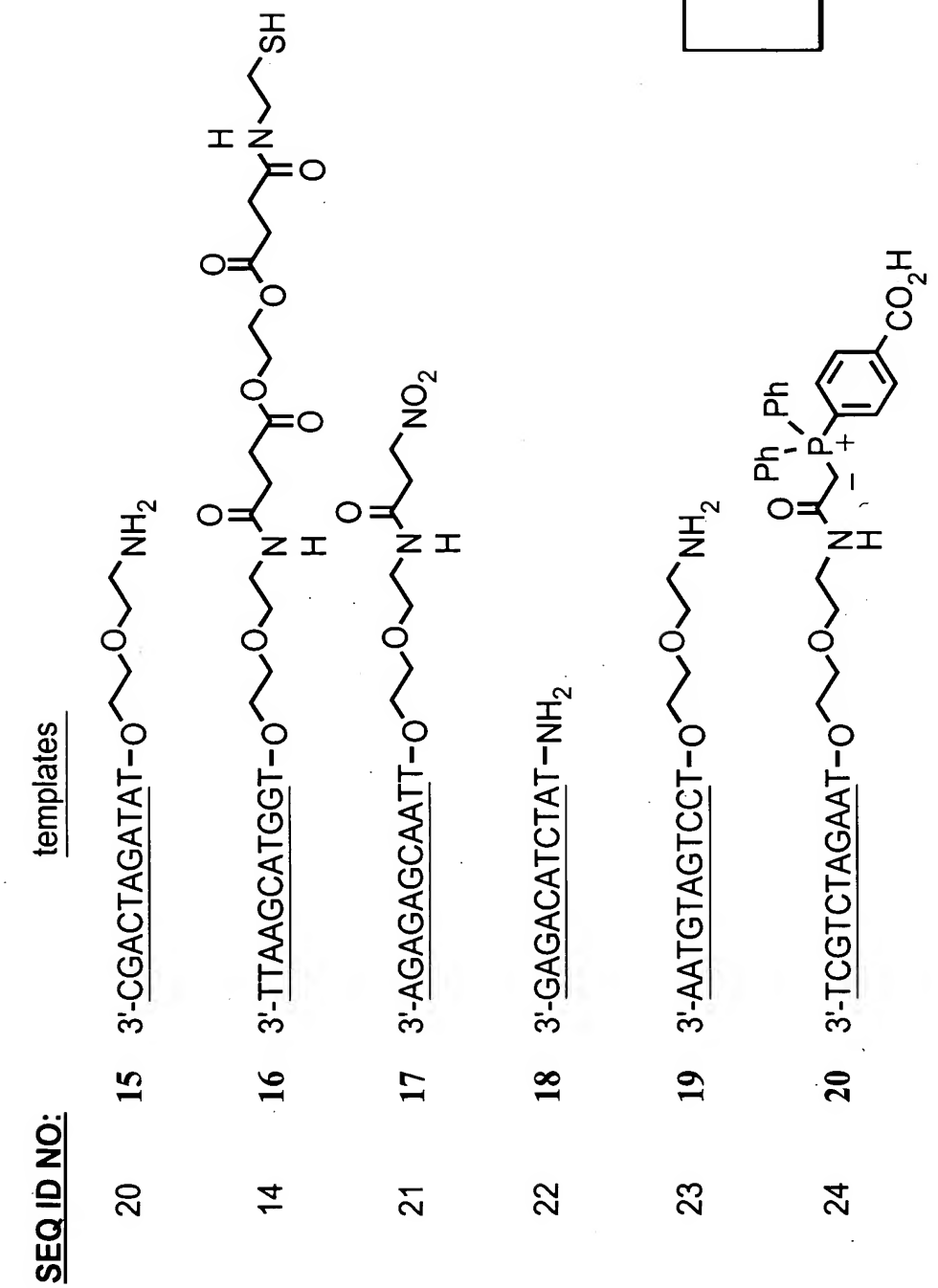


FIG. 47A

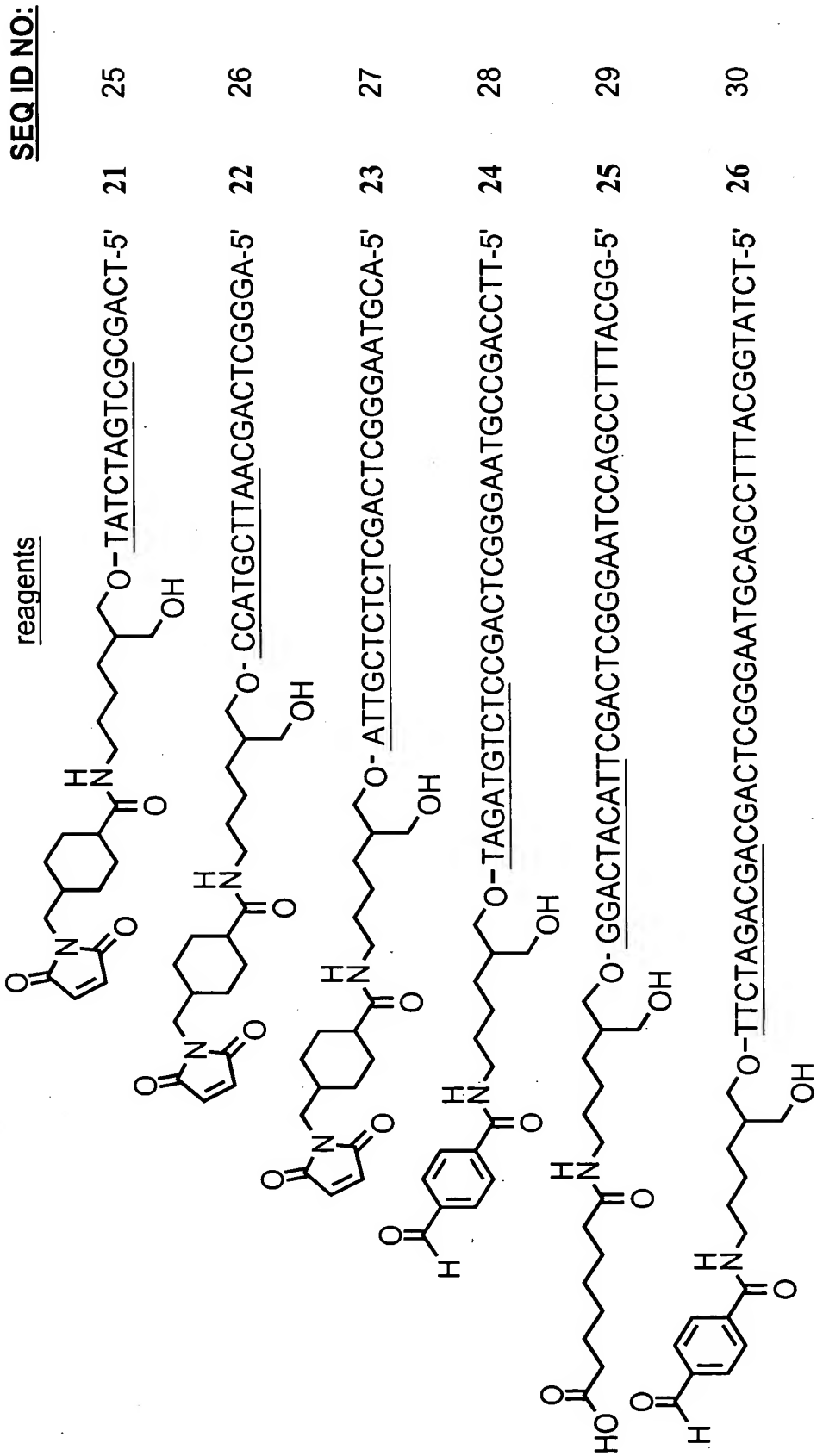

SEQ ID NO:
reagents
21
25
O-TATCTAGTCGCGACT-5'
22
26
O-CCATGCTTAACGACTCGGGA-5'
23
27
O-ATTGCTCTCTCGACTCGGGAATGCA-5'
24
28
O-TAGATGTCTCCGACTCGGGAATGCCGACCTT-5'
25
29
O-GGACTACATTCGACTCGGGAATCCAGCCTTTACGG-5'
26
30
O-TTCTAGACGACTCGGGAATGCAGCCTTTACGGTATCT-5'

FIG. 47B

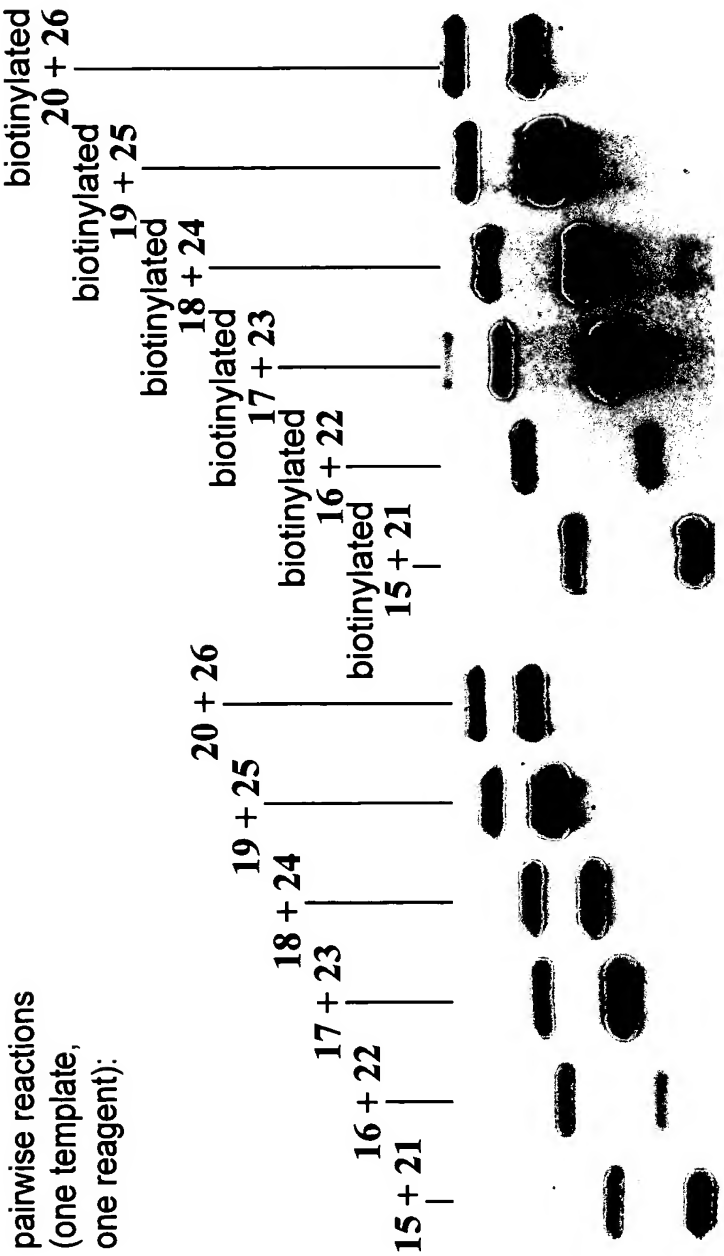
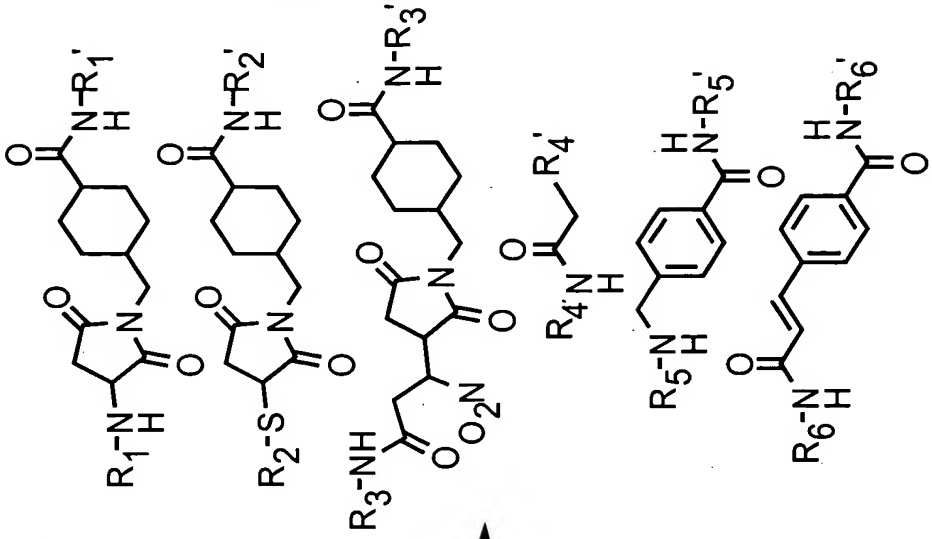

pairwise reactions
(one template,
one reagent):
15 + 21
16 + 22
17 + 23
18 + 24
19 + 25
20 + 26
biotinylated
15 + 21
biotinylated
16 + 22
biotinylated
17 + 23
biotinylated
18 + 24
biotinylated
19 + 25
biotinylated
20 + 26

FIG. 47C

FIG. 48A



12 reactants in one solution

EDC, sulfo-NHS, $NaBH_3CN$

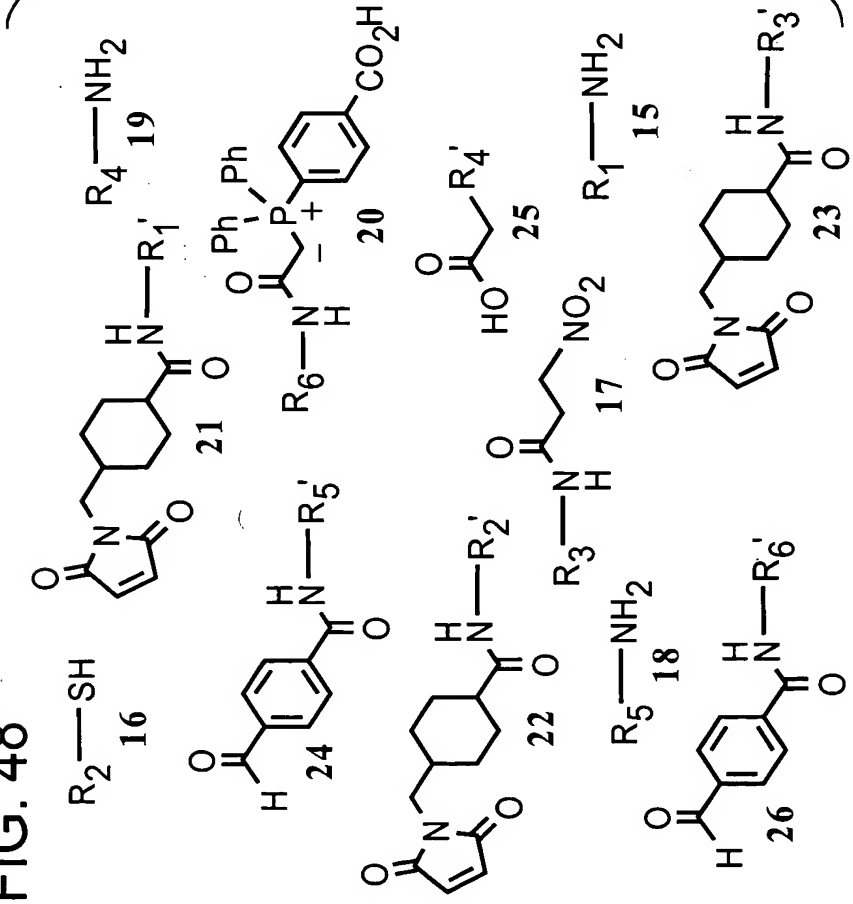


FIG. 48

FIG. 48A	FIG. 48B

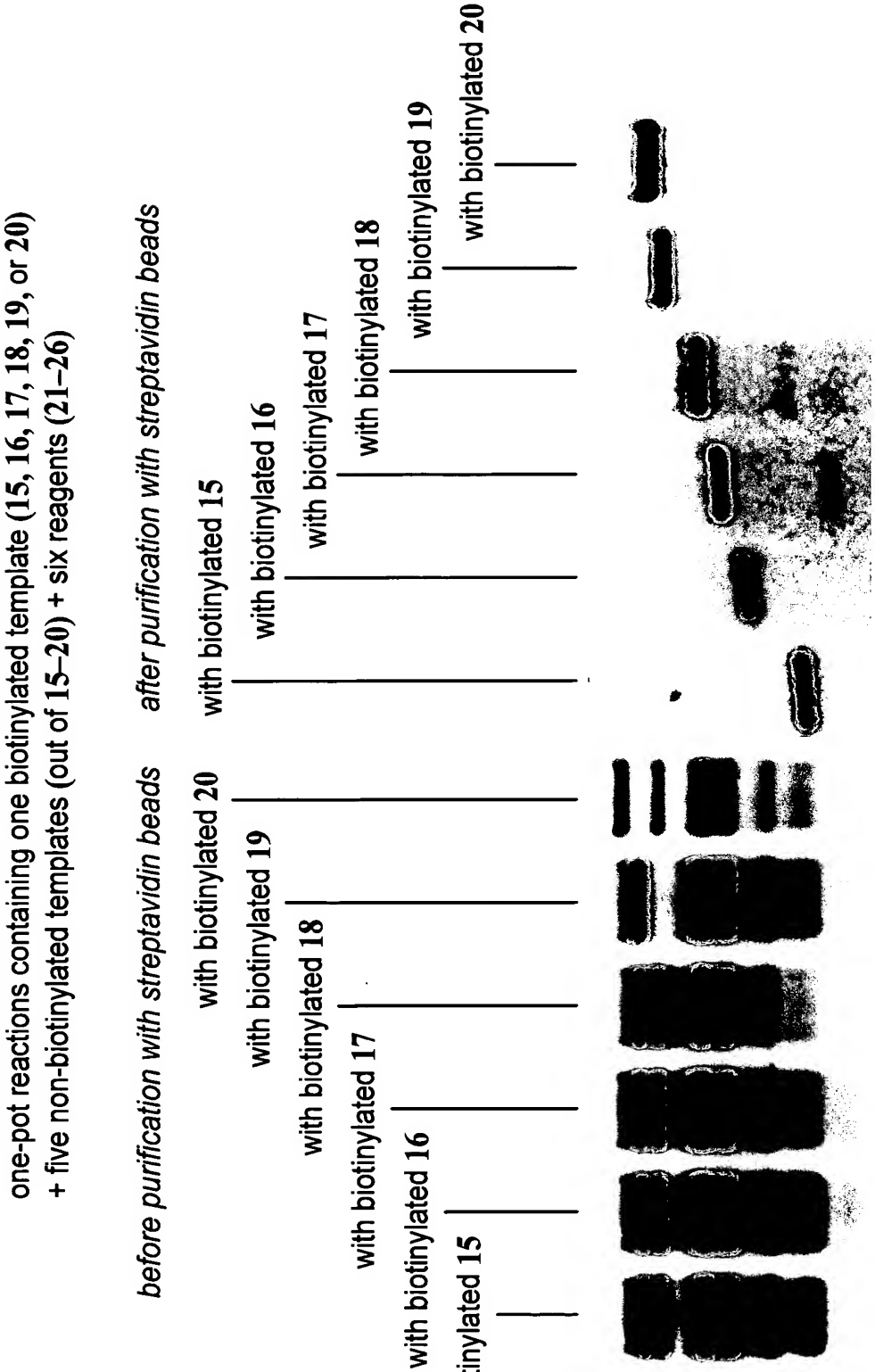

one-pot reactions containing one biotinylated template (15, 16, 17, 18, 19, or 20)
+ five non-biotinylated templates (out of 15–20) + six reagents (21–26)
before purification with streptavidin beads
with biotinylated 15
with biotinylated 16
with biotinylated 17
with biotinylated 18
with biotinylated 19
with biotinylated 20
after purification with streptavidin beads
with biotinylated 15
with biotinylated 16
with biotinylated 17
with biotinylated 18
with biotinylated 19
with biotinylated 20

FIG. 48B

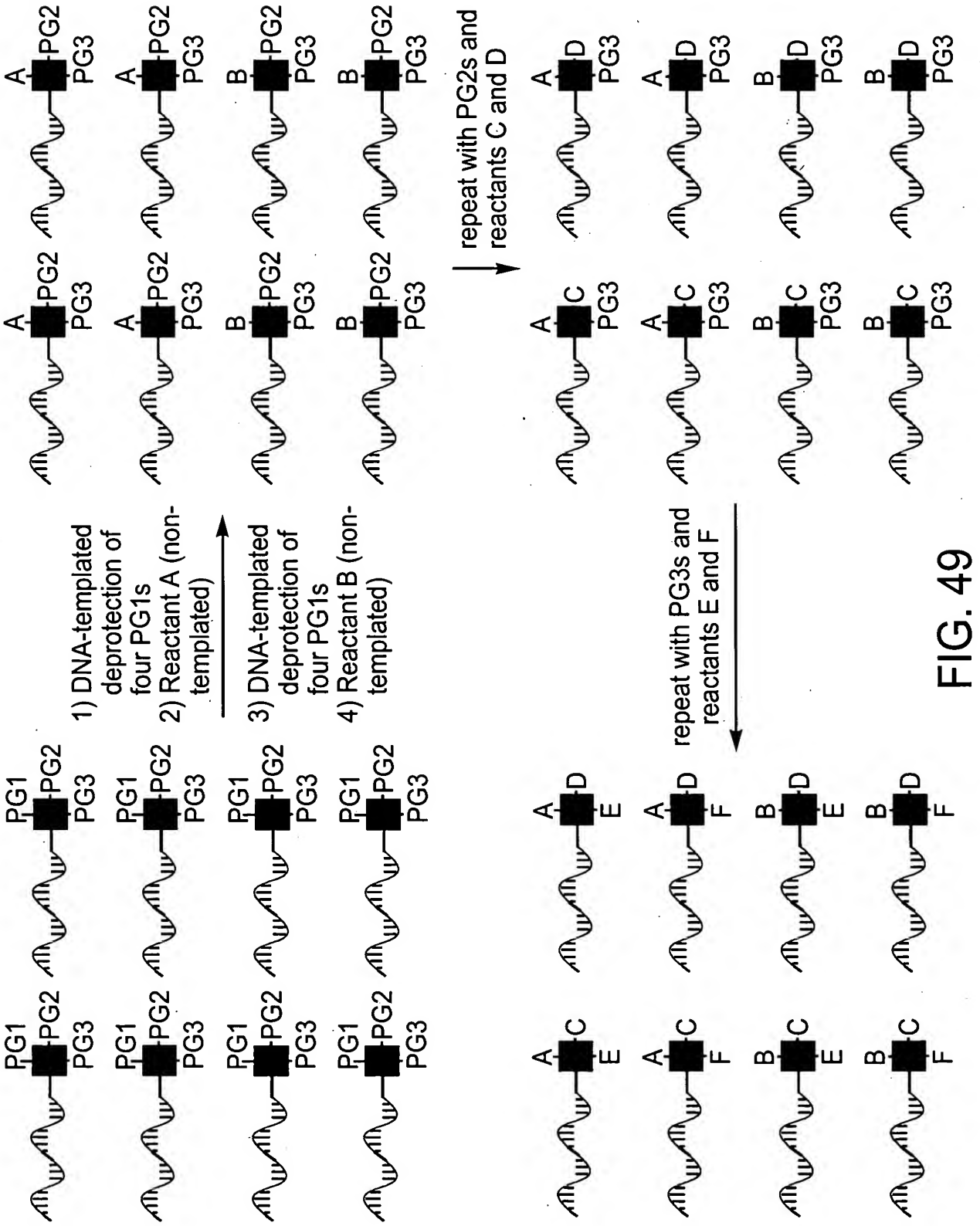

1) DNA-templated deprotection of four PG1s
2) Reactant A (non-templated)
3) DNA-templated deprotection of four PG1s
4) Reactant B (non-templated)
repeat with PG2s and reactants C and D
repeat with PG3s and reactants E and F
PG1
PG2
PG3
A
B
C
D
E
F

FIG. 49

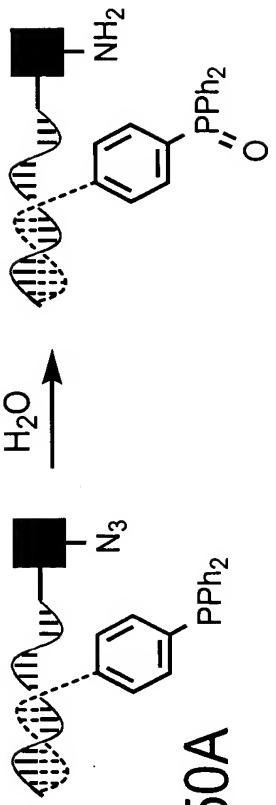


FIG. 50A

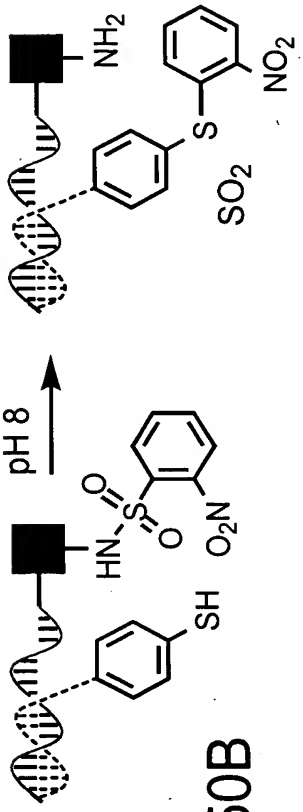


FIG. 50B

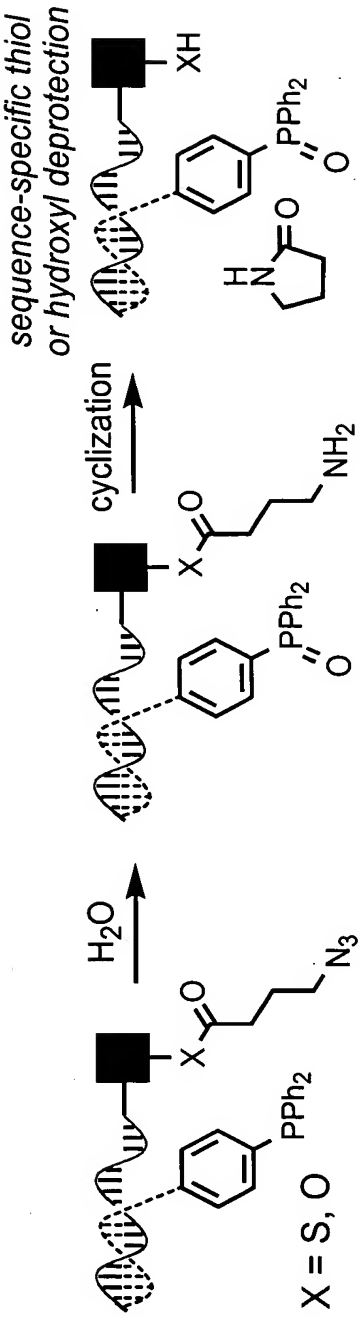


FIG. 50C

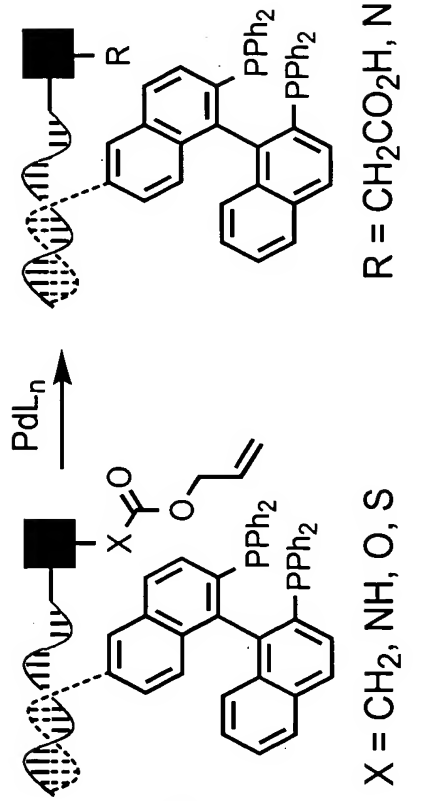


FIG. 50D

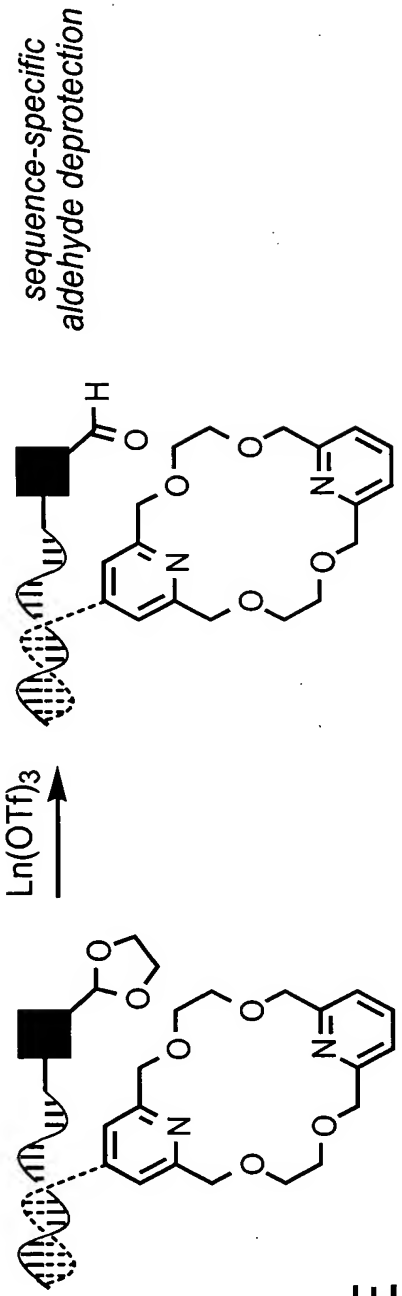


FIG. 50E

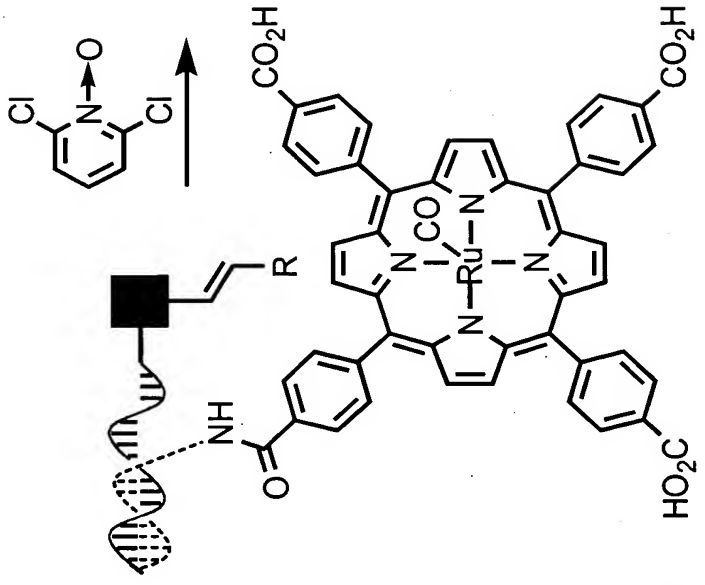


FIG. 51A

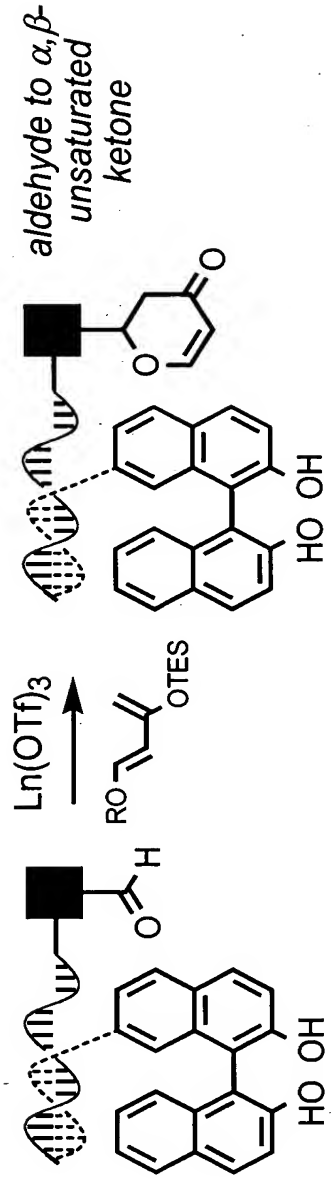


FIG. 51B

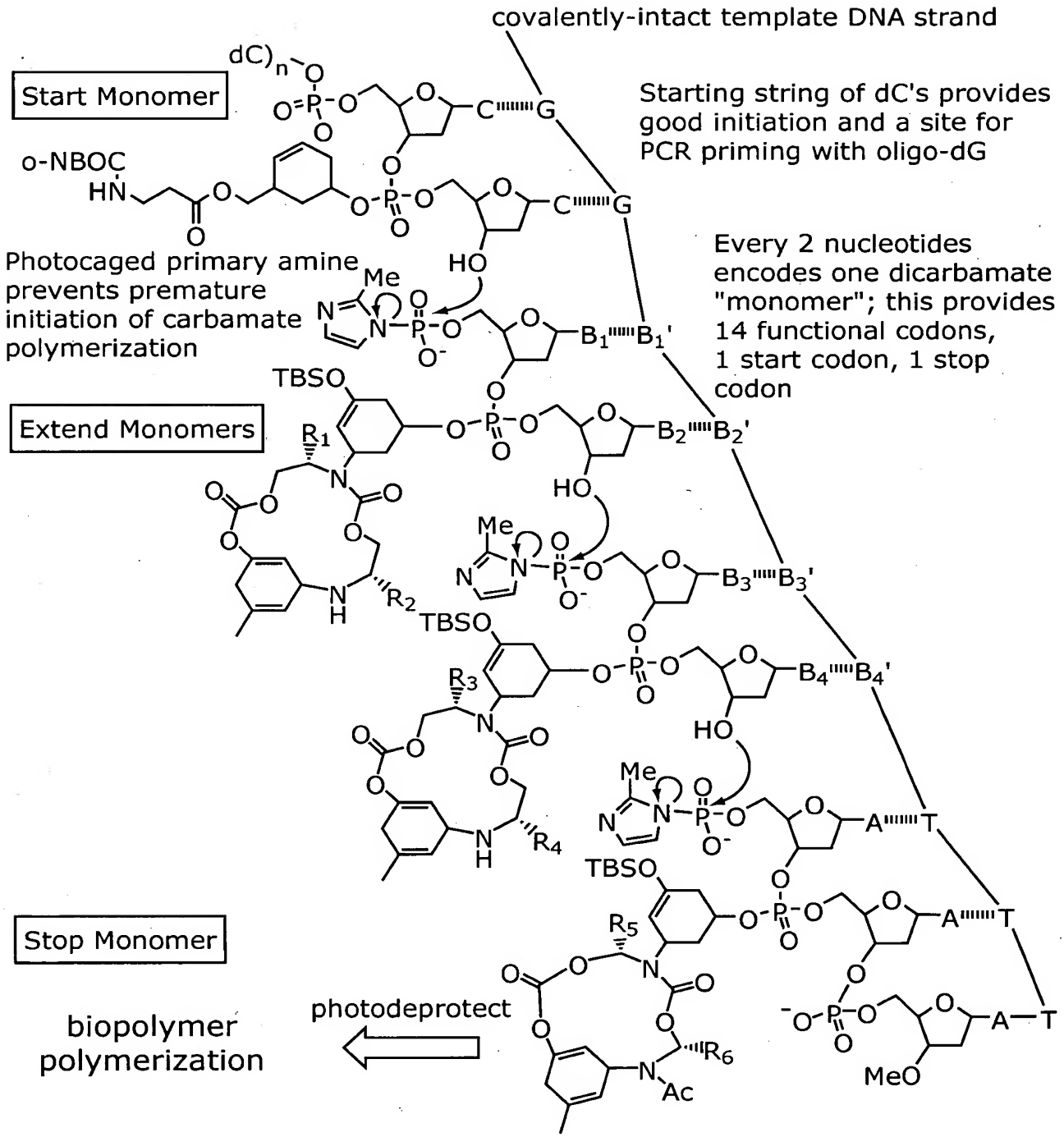

covalently-intact template DNA strand
Start Monomer
Starting string of dC's provides good initiation and a site for PCR priming with oligo-dG
Photocaged primary amine prevents premature initiation of carbamate polymerization
Every 2 nucleotides encodes one dicarbamate "monomer"; this provides 14 functional codons, 1 start codon, 1 stop codon
Extend Monomers
Stop Monomer
photodeprotect
biopolymer polymerization

FIG. 52

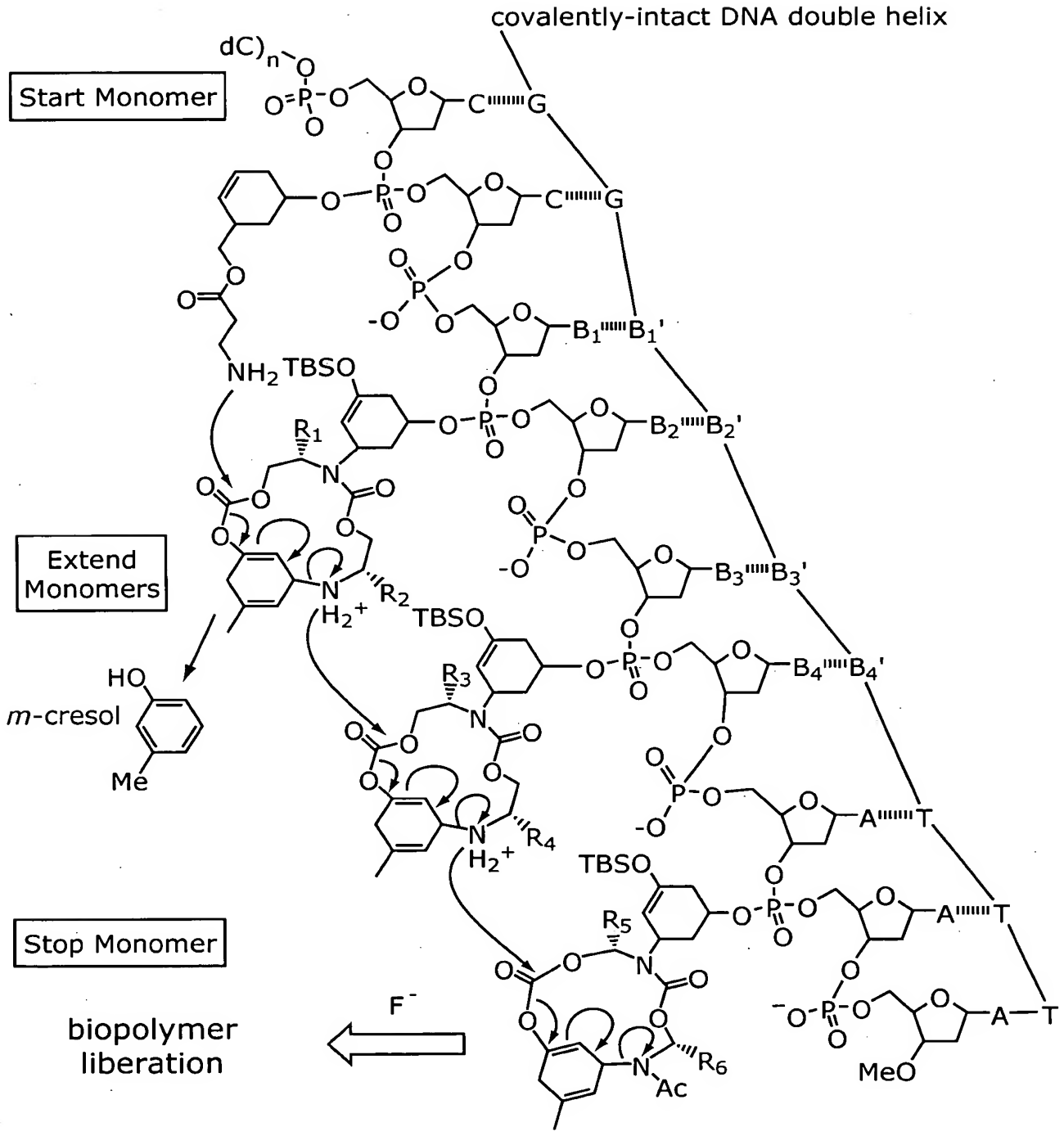

covalently-intact DNA double helix
Start Monomer
Extend Monomers
Stop Monomer
biopolymer liberation
F⁻
m-cresol
HO
Me
TBSO
MeO
Ac

FIG. 53

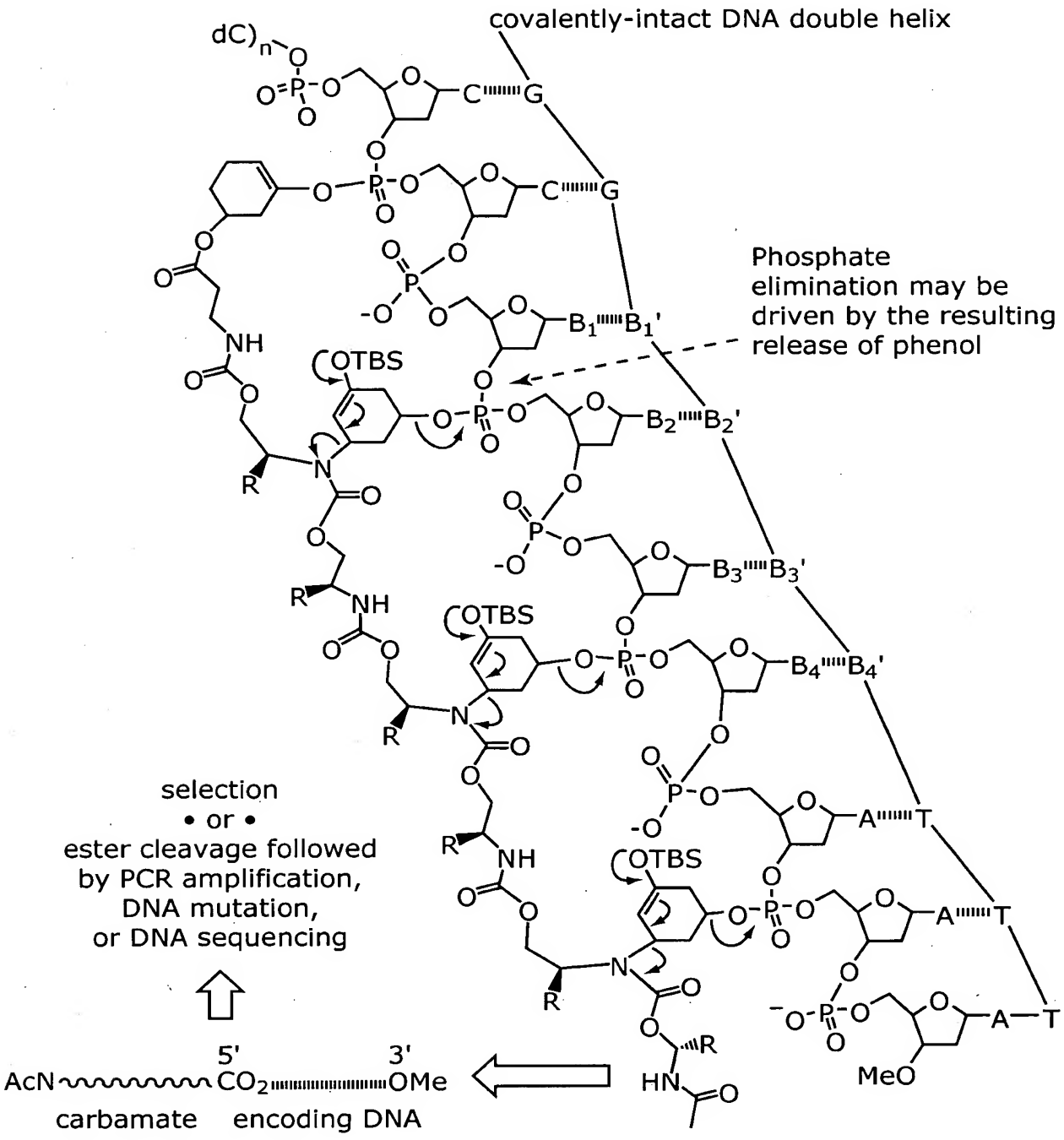

covalently-intact DNA double helix
Phosphate elimination may be driven by the resulting release of phenol
selection
• or •
ester cleavage followed by PCR amplification, DNA mutation, or DNA sequencing
5'
3'
AcN
CO2
OMe
carbamate
encoding DNA
OTBS
MeO

FIG. 54

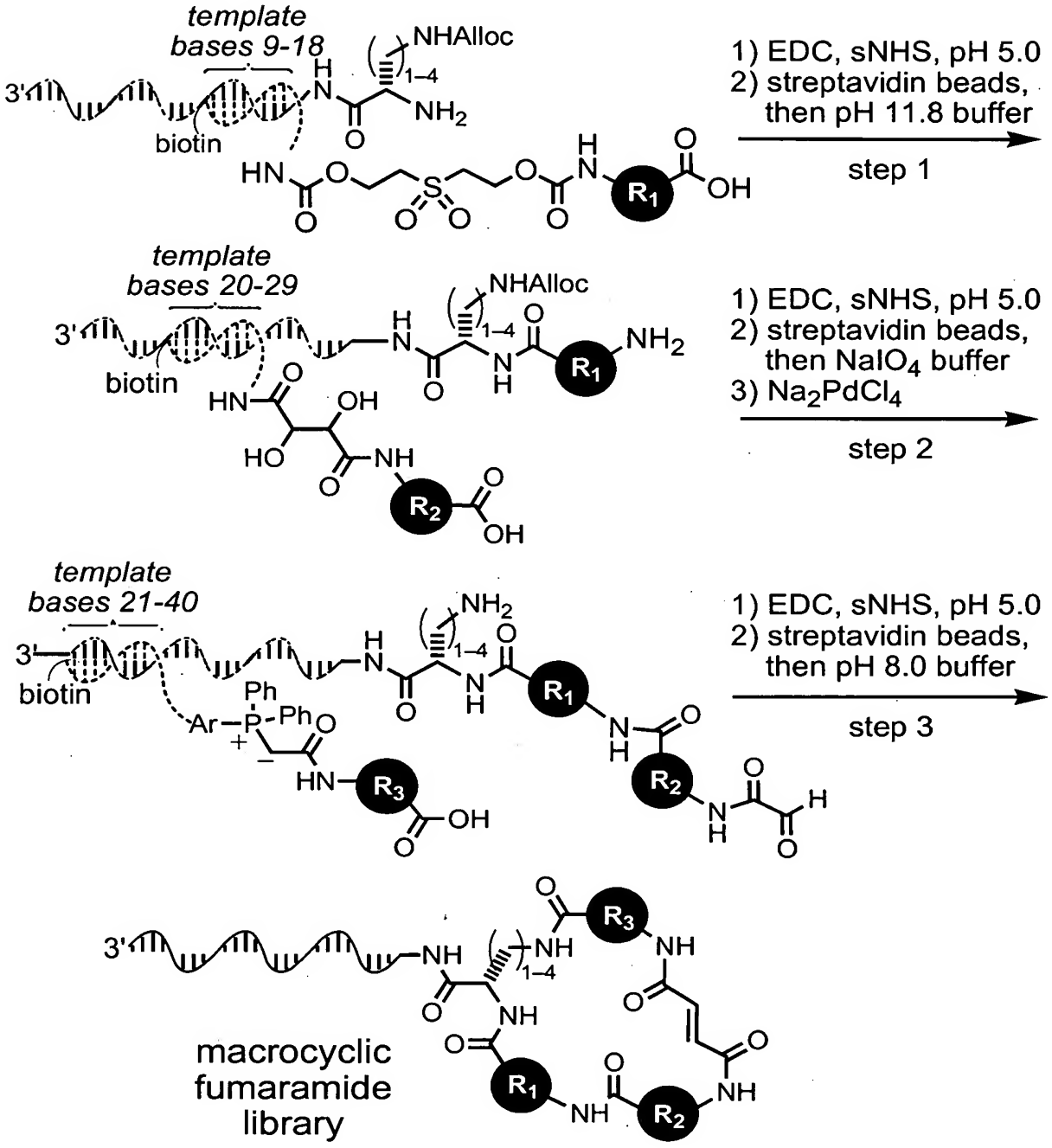

template
bases 9-18
NHAlloc
1–4
3'
biotin
1) EDC, sNHS, pH 5.0
2) streptavidin beads,
then pH 11.8 buffer
step 1
template
bases 20-29
NHAlloc
1–4
3'
biotin
1) EDC, sNHS, pH 5.0
2) streptavidin beads,
then $NaIO_4$ buffer
3) Na_2PdCl_4
step 2
template
bases 21-40
3'
biotin
1) EDC, sNHS, pH 5.0
2) streptavidin beads,
then pH 8.0 buffer
step 3
3'
macrocyclic
fumaramide
library

FIG. 55

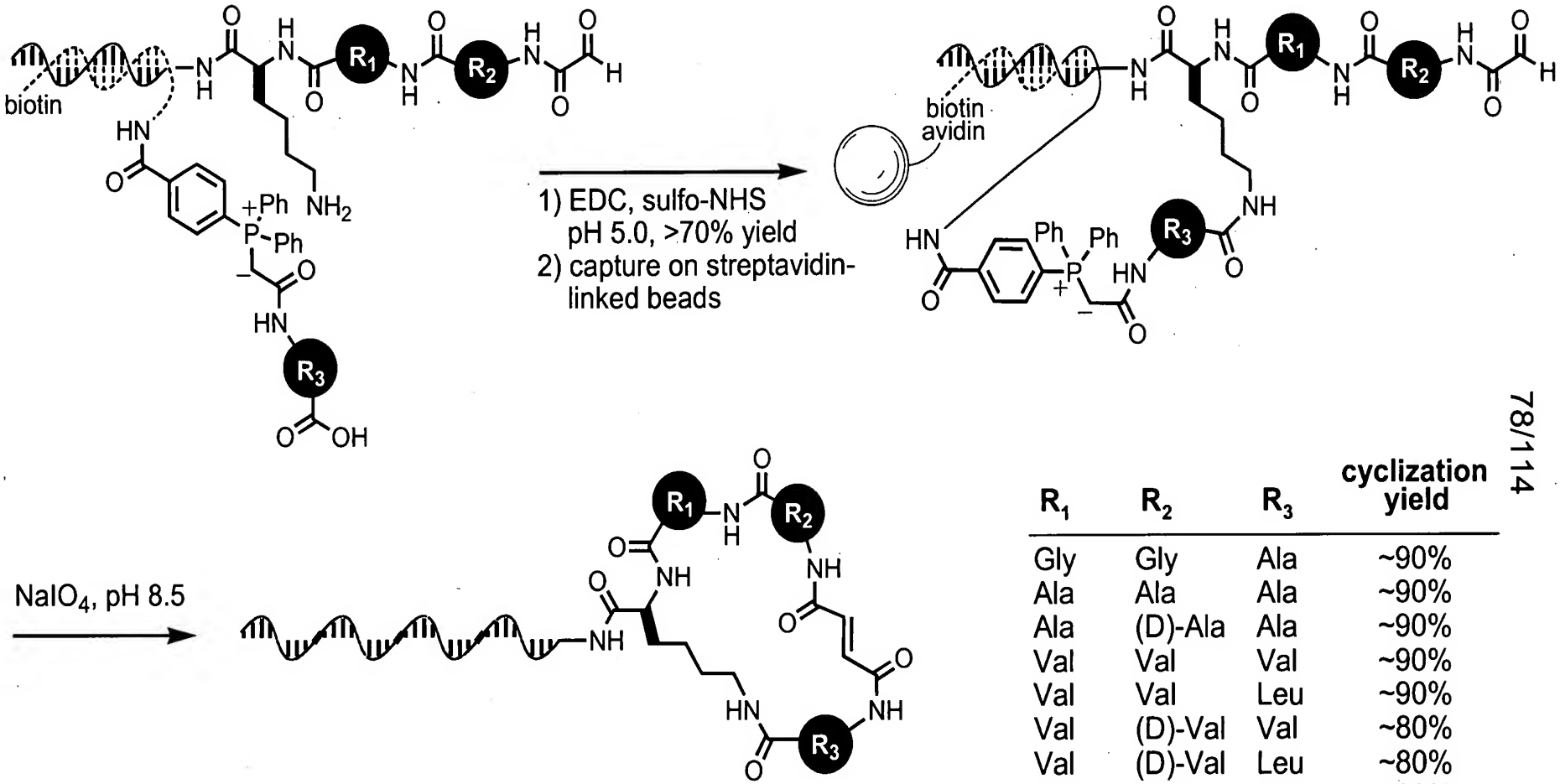


FIG. 56

R_1	R_2	R_3	cyclization yield
Gly	Gly	Ala	~90%
Ala	Ala	Ala	~90%
Ala	(D)-Ala	Ala	~90%
Val	Val	Val	~90%
Val	Val	Leu	~90%
Val	(D)-Val	Val	~80%
Val	(D)-Val	Leu	~80%
Phe	Phe	Ala	~80%
Phe	Phe	Leu	~80%
GABA	GABA	β-Ala	~80%
Phe	Phe	Phe	~60%

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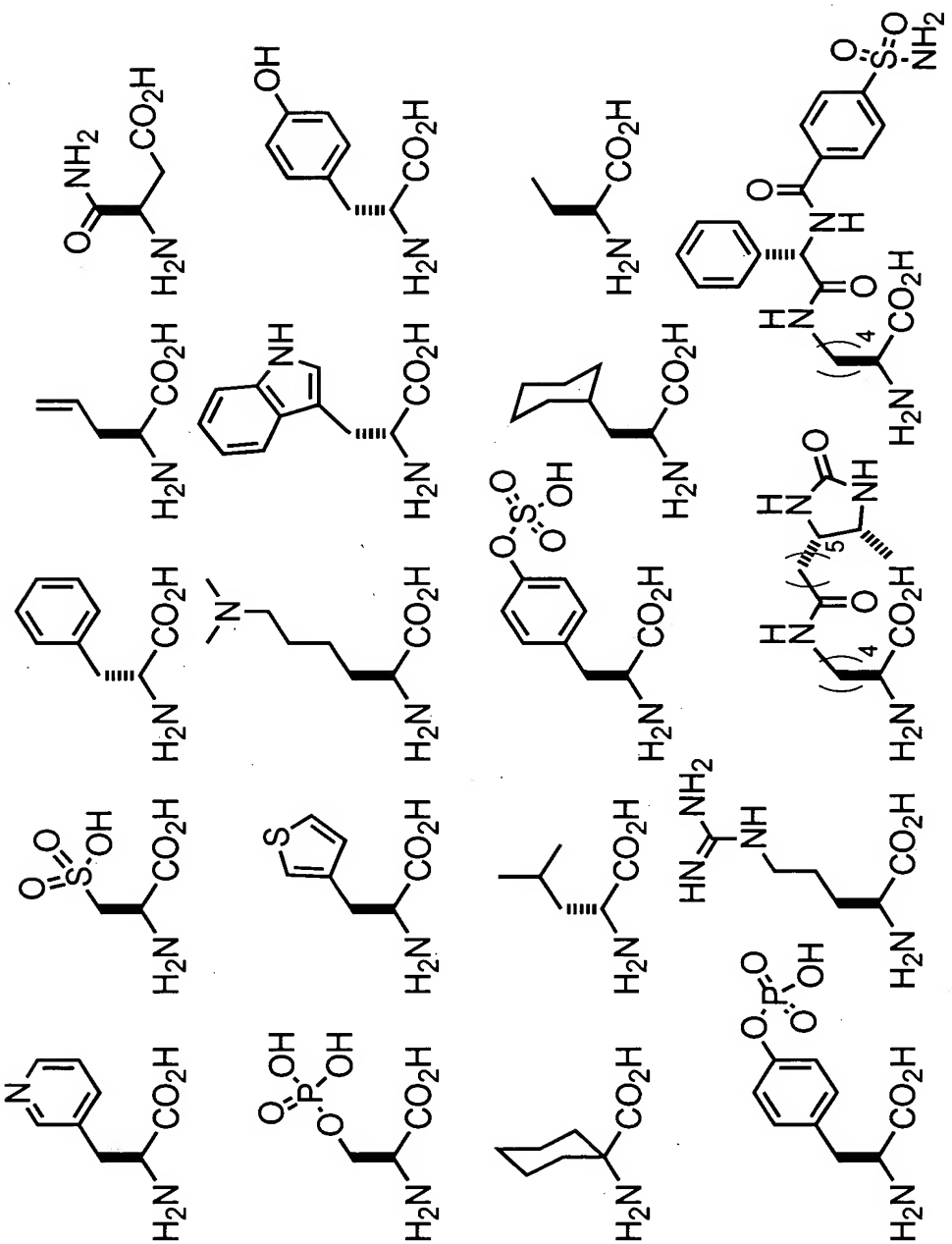


FIG. 57

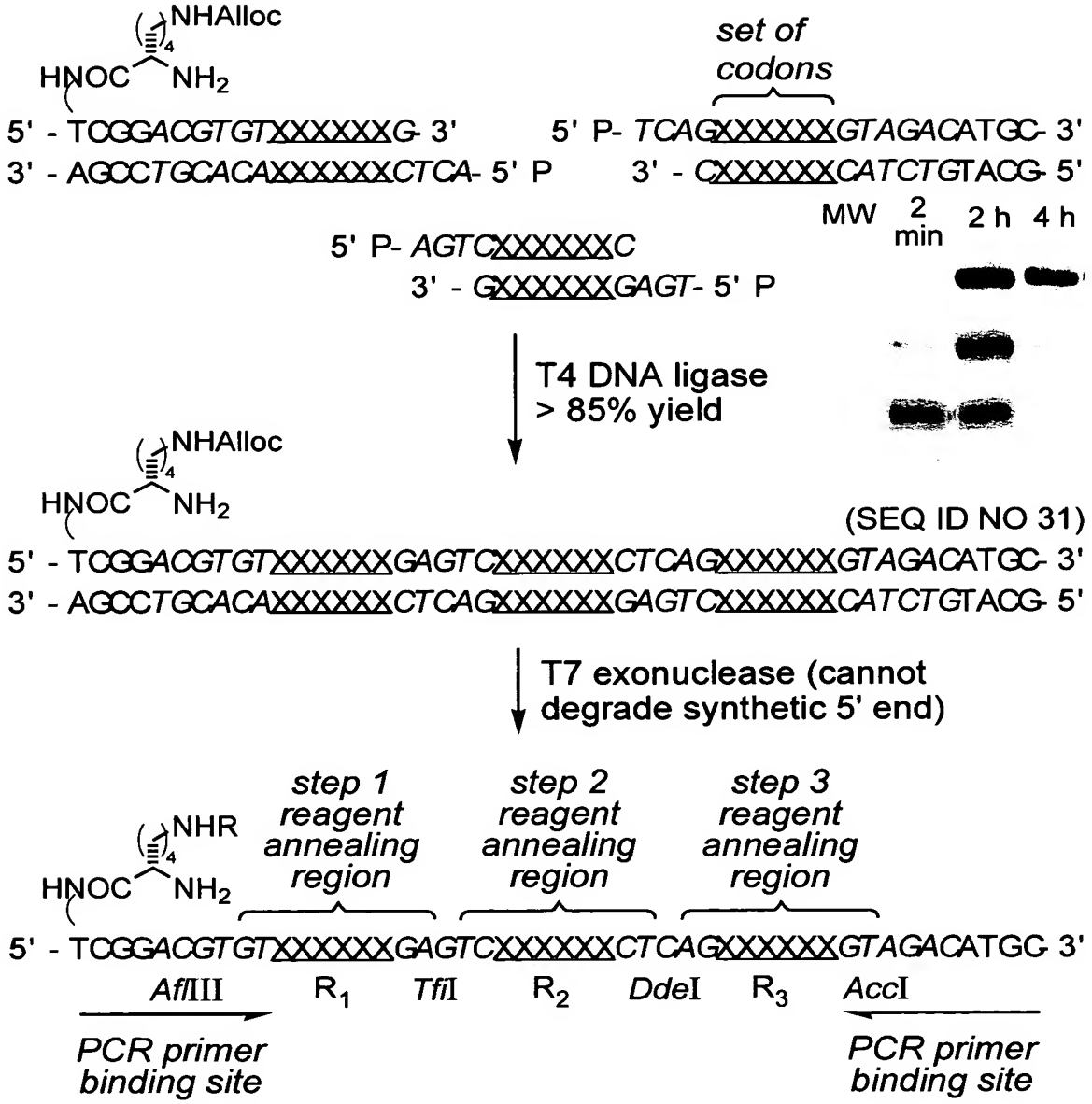

NHAlloc
HNOC
NH2
set of codons
5' - TCGGACGTGTXXXXXXG- 3'
3' - AGCCTGCACAXXXXXXCTCA- 5' P
5' P- TCAGXXXXXXGTAGACATGC- 3'
3' - CXXXXXXCATCTGTACG- 5'
MW
2 min
2 h
4 h
5' P- AGTCXXXXXXC
3' - GXXXXXXGAGT- 5' P
T4 DNA ligase
> 85% yield
NHAlloc
HNOC
NH2
(SEQ ID NO 31)
5' - TCGGACGTGTXXXXXXGAGTCXXXXXXCTCAGXXXXXXGTAGACATGC- 3'
3' - AGCCTGCACAXXXXXXCTCAGXXXXXXGAGTCXXXXXXCATCTGTACG- 5'
T7 exonuclease (cannot degrade synthetic 5' end)
NHR
HNOC
NH2
step 1 reagent annealing region
step 2 reagent annealing region
step 3 reagent annealing region
5' - TCGGACGTGTXXXXXXGAGTCXXXXXXCTCAGXXXXXXGTAGACATGC- 3'
AflIII
R1
TfiI
R2
DdeI
R3
AccI
PCR primer binding site
PCR primer binding site

FIG. 58

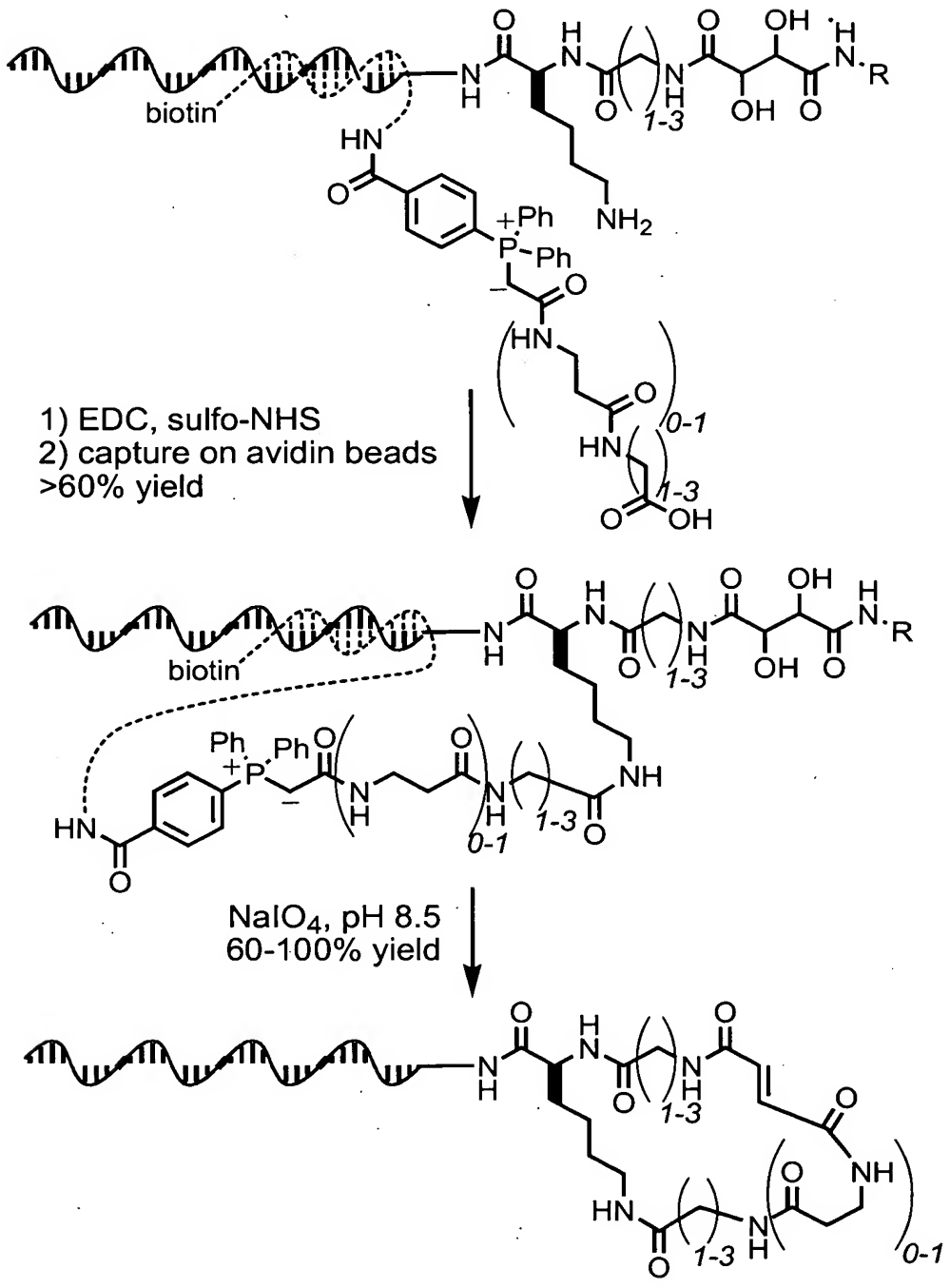
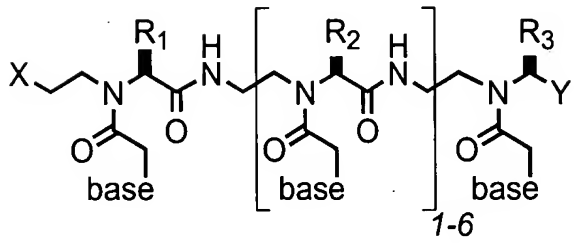

biotin
1) EDC, sulfo-NHS
2) capture on avidin beads
>60% yield
biotin
NaIO4, pH 8.5
60-100% yield

FIG. 59



X, Y = groups for coupling (see Fig. 1)

R =

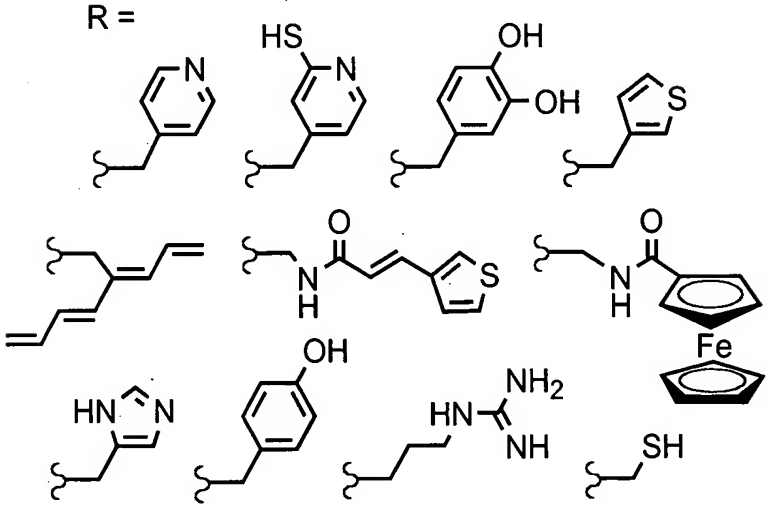


FIG. 60

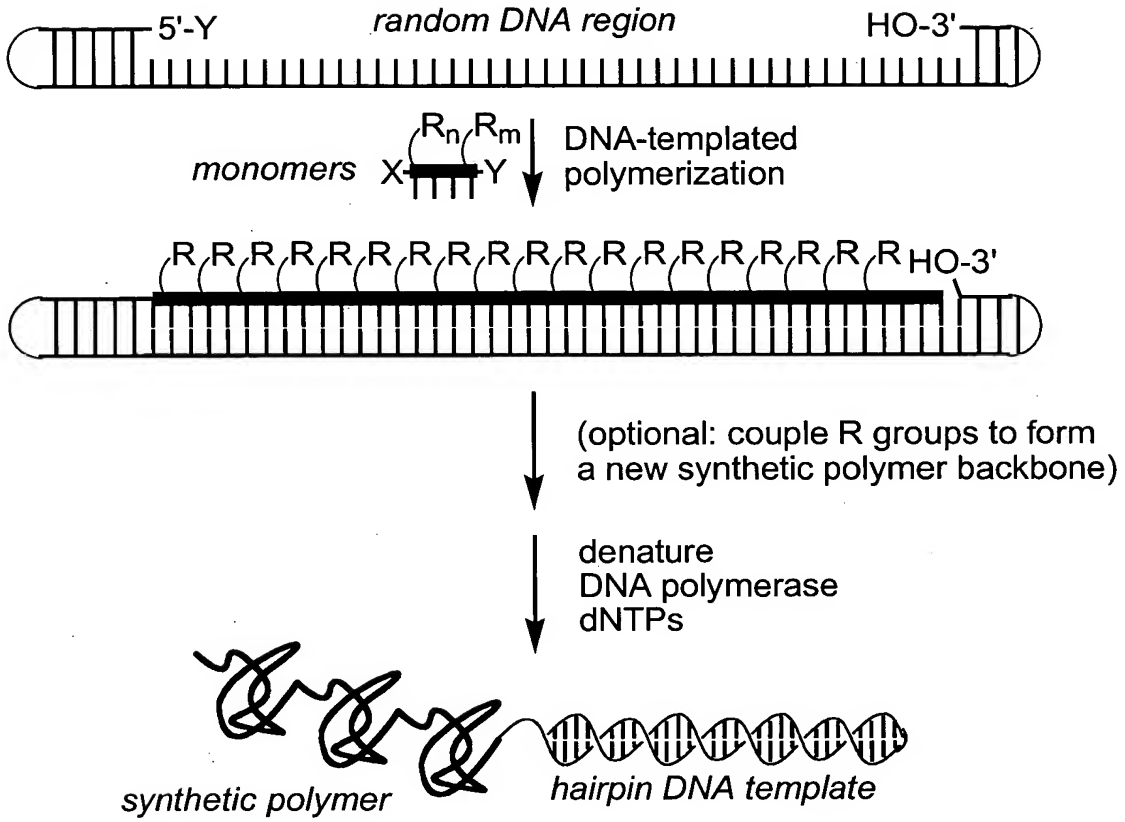

5'-Y
random DNA region
HO-3'
monomers
R_n R_m
X
Y
DNA-templated
polymerization
R R R R R R R R R R R R R R R R R R HO-3'
(optional: couple R groups to form
a new synthetic polymer backbone)
denature
DNA polymerase
dNTPs
synthetic polymer
hairpin DNA template

FIG. 61

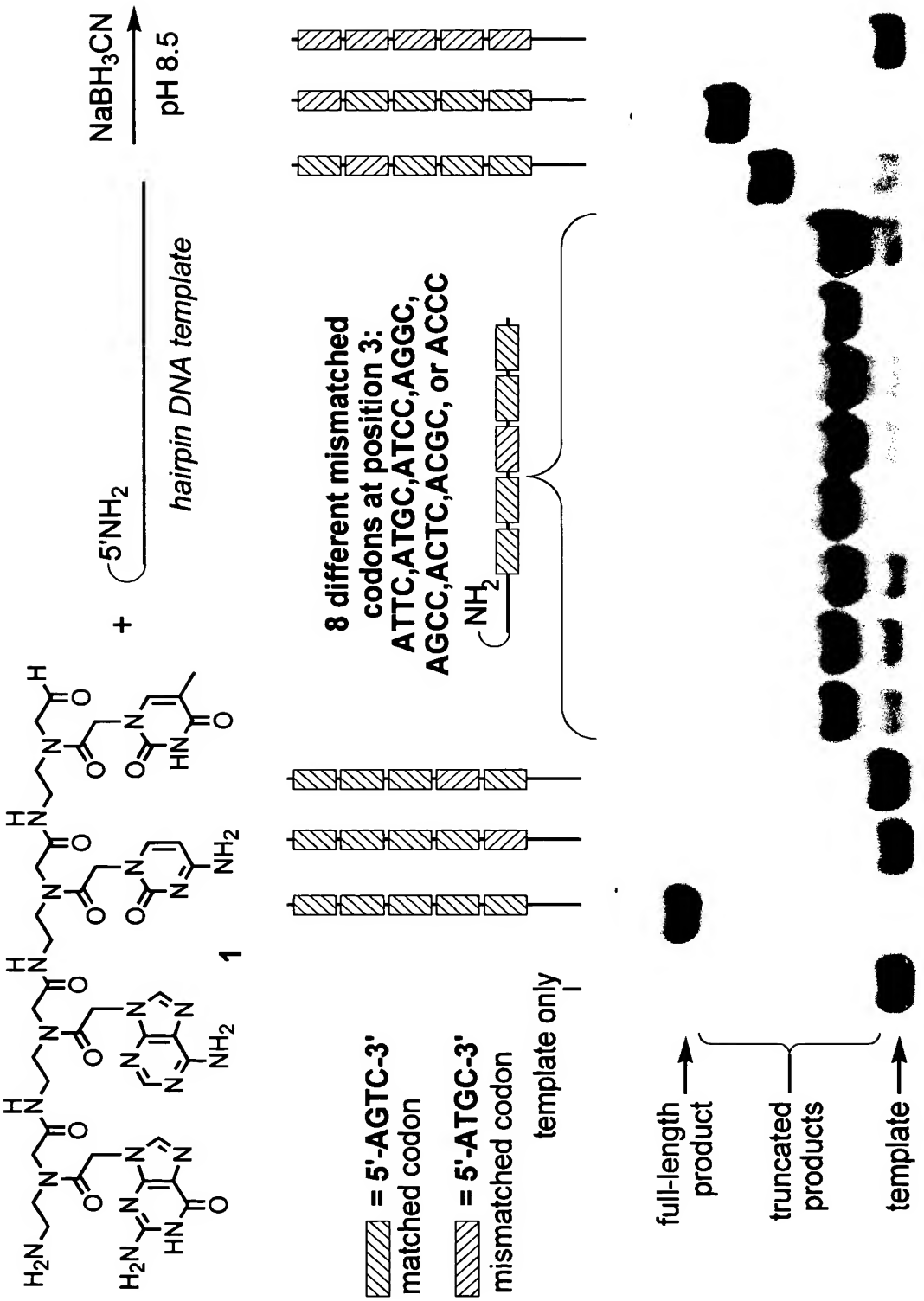

NaBH3CN
pH 8.5
5'NH2
hairpin DNA template
+
1
8 different mismatched codons at position 3: ATTC,ATGC,ATCC,AGGC, AGCC,ACTC,ACGC, or ACCC
NH2
= 5'-AGTC-3' matched codon
= 5'-ATGC-3' mismatched codon
template only
full-length product
truncated products
template

FIG. 62

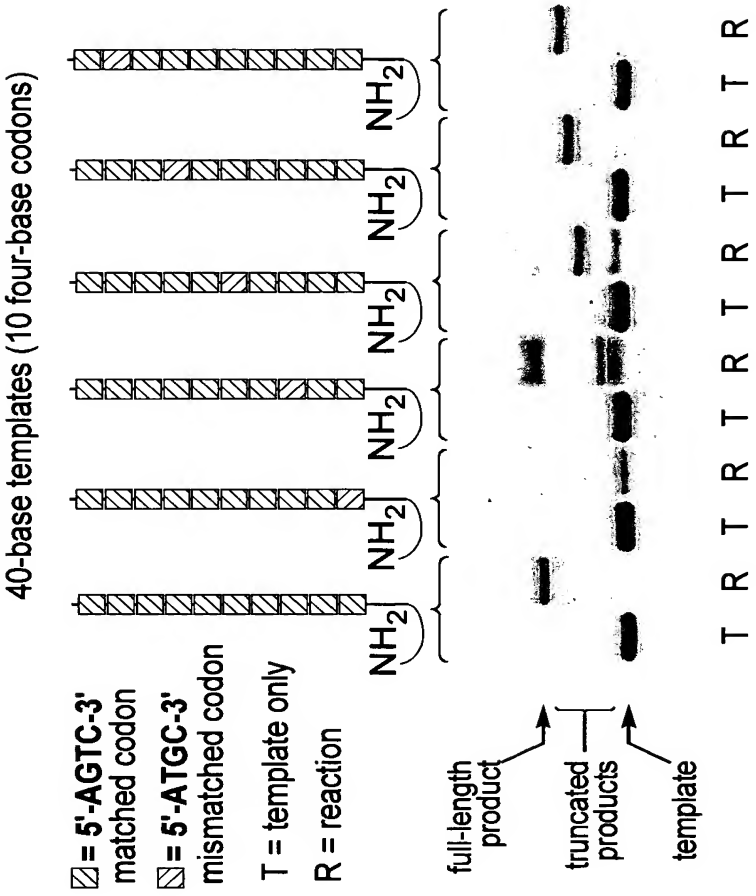

40-base templates (10 four-base codons)
= 5'-AGTC-3' matched codon
= 5'-ATGC-3' mismatched codon
T = template only
R = reaction
NH2
full-length product
truncated products
template
T R T R T R T R T R

FIG. 63

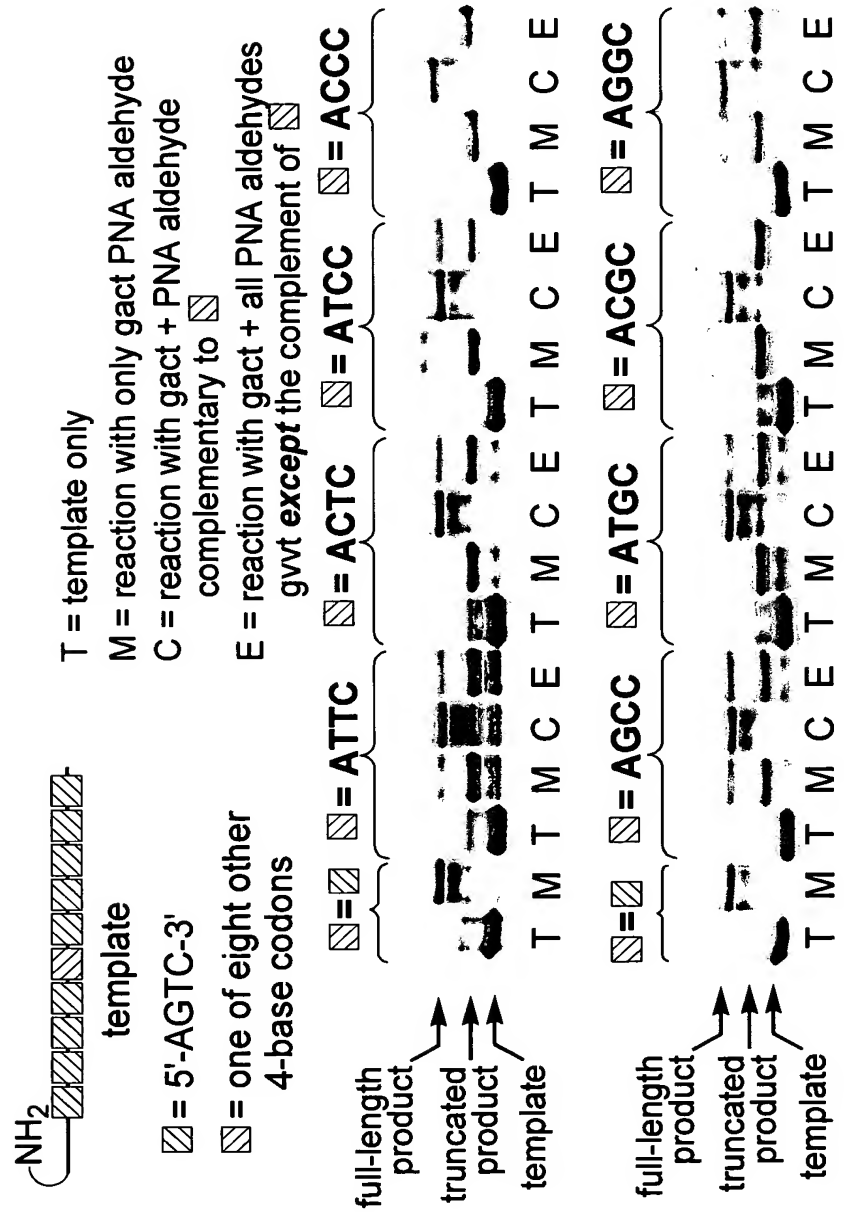
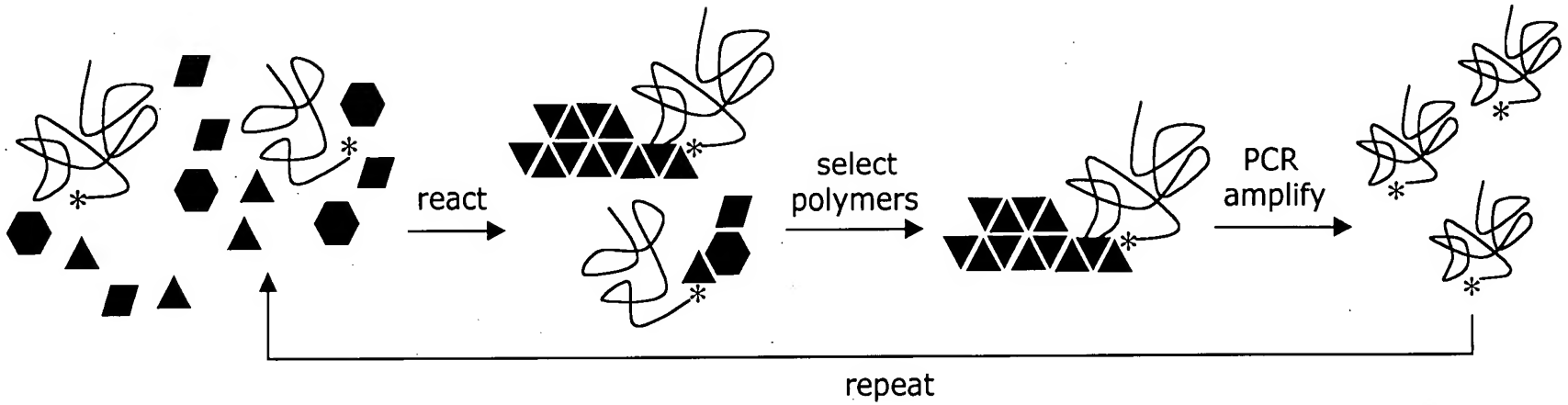

NH2
template
▨ = 5'-AGTC-3'
▨ = one of eight other 4-base codons
T = template only
M = reaction with only gact PNA aldehyde
C = reaction with gact + PNA aldehyde complementary to ▨
E = reaction with gact + all PNA aldehydes gvvt **except** the complement of ▨
▨=▨ ▨= ATTC ▨= ACTC ▨= ATCC ▨= ACCC
full-length product
truncated product
template
T M T M C E T M C E T M C E
▨=▨ ▨= AGCC ▨= ATGC ▨= ACGC ▨= AGGC
full-length product
truncated product
template
T M T M C E T M C E T M C E

FIG. 64

Evolving Plastics

How can amplifiable information be translated into materials with specific properties (e.g., plastics)?

- Nucleic acids can fold into complex structures



Requirements:

- -Linkage between information and product: need living polymerization
- -Selection for desired materials: gel electrophoresis, sedimentation, mechanical sorting, solvent partitioning
- -Chemical compatibility with DNA: stability in water

FIG. 65A

Title: Evolving New Molecular Function
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Evolving Plastics

Ring-opening metathesis polymerization (ROMP, R. Grubbs) is mediated by a ruthenium catalyst

ROMP is aqueous-compatible and is a living polymerization

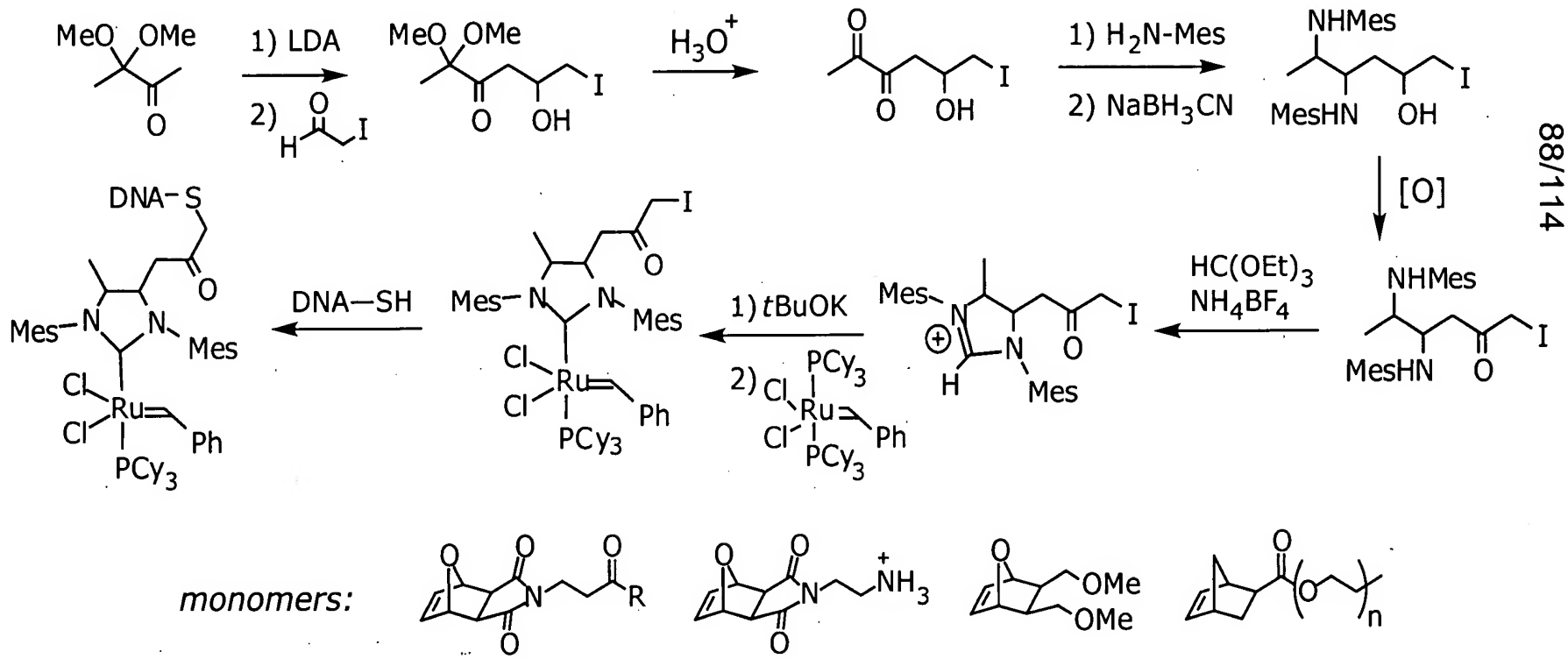

FIG. 65B

Title: Evolving New Molecular Function
Inventor: Liu *et al.*
Serial No.: 10/643,752
Atty Docket No.: LS5-001 (9792/5)
Atty/Agent: Brian A. Fairchild
Express Mail Label No.: EL932952135US
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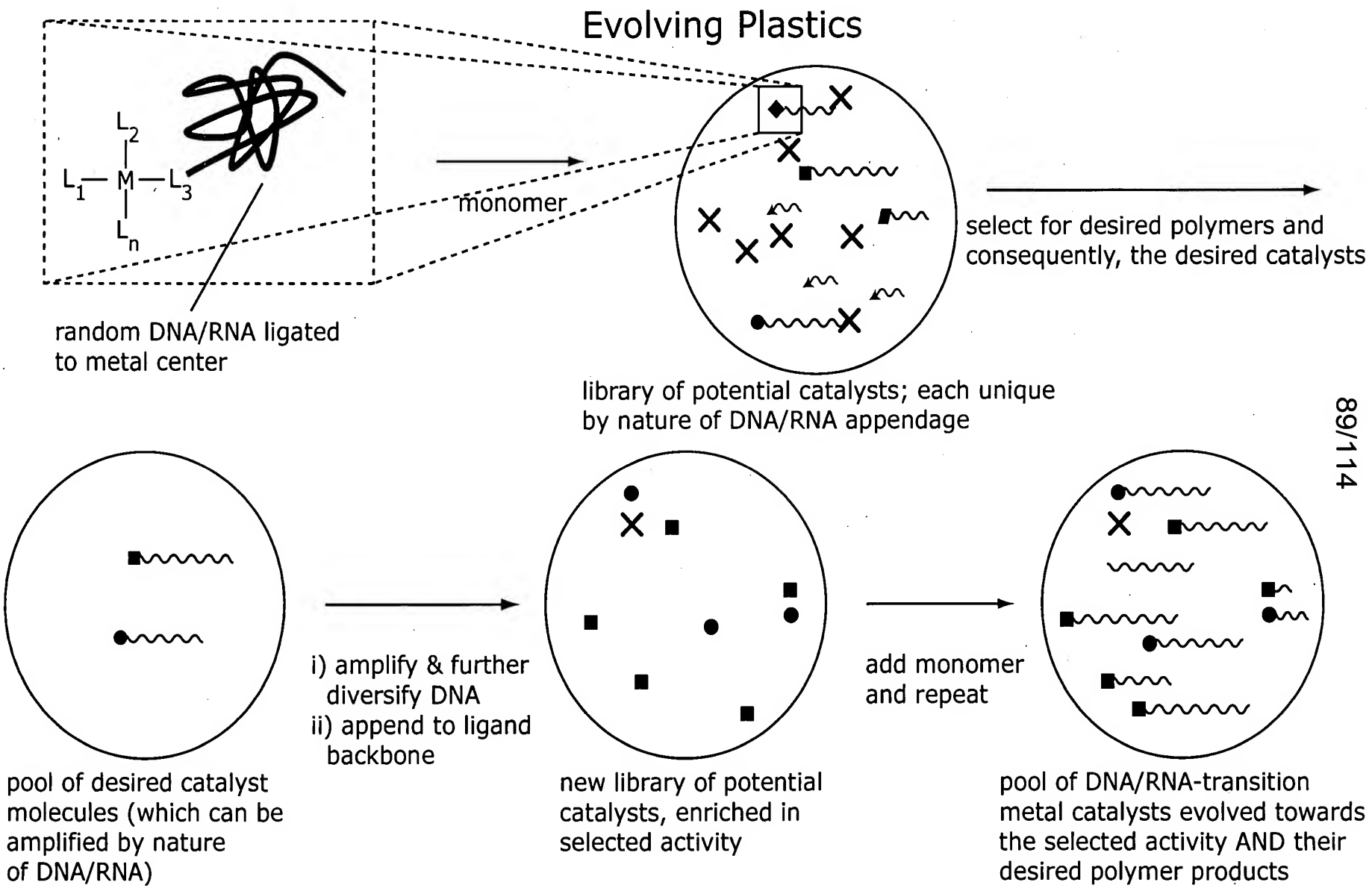

FIG. 66

Title: Evolving New Molecular Function
Inventor: Liu *et al.*
Serial No.: 10/643,752
Atty Docket No.: LS5-001 (9792/5)
Atty/Agent: Brian A. Fairchild
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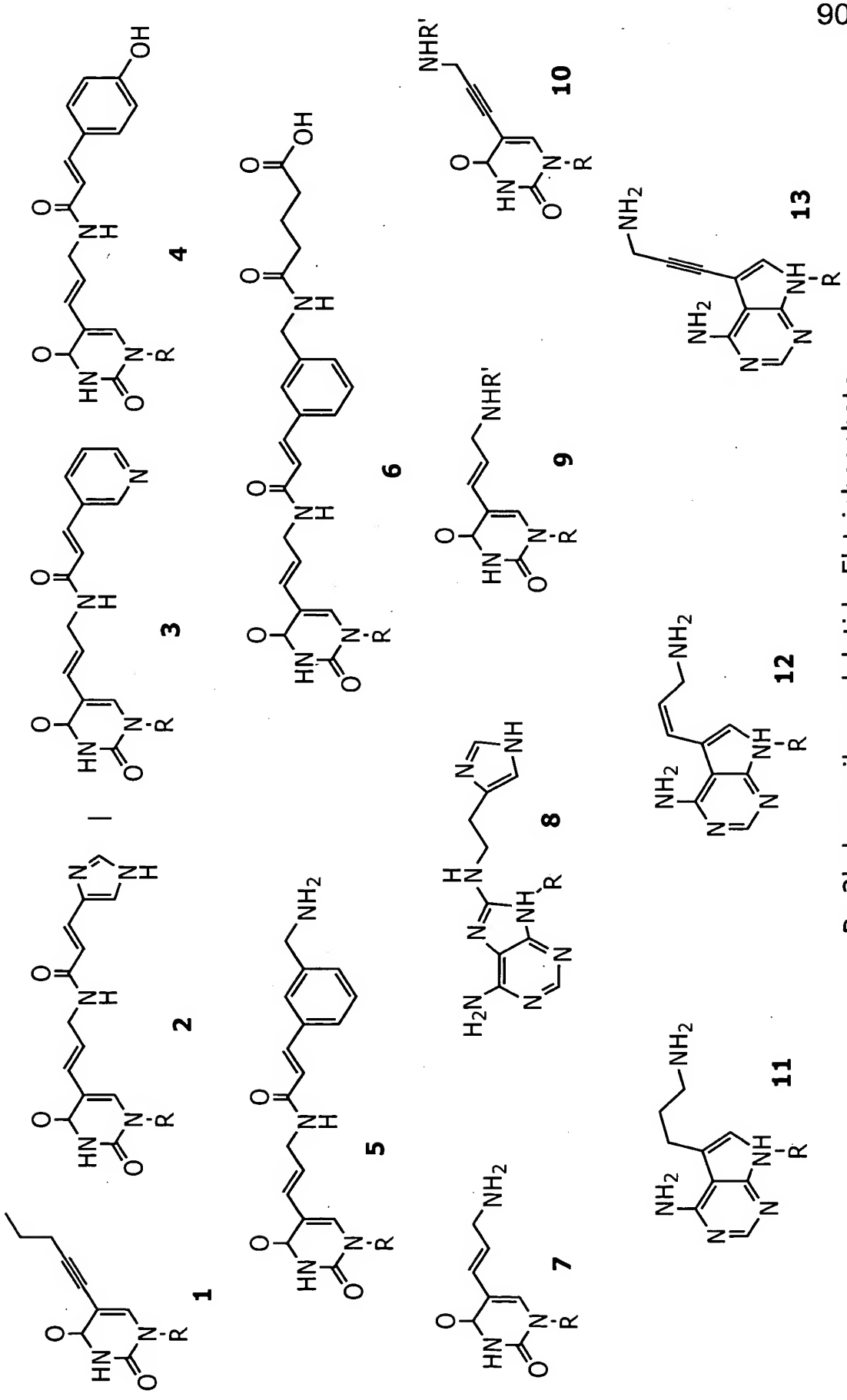
1
2
3
4
5
6
7
8
9
10
11
12
13
R=2'-deoxyribonuclelotide 5'-triphosphate

FIG. 67

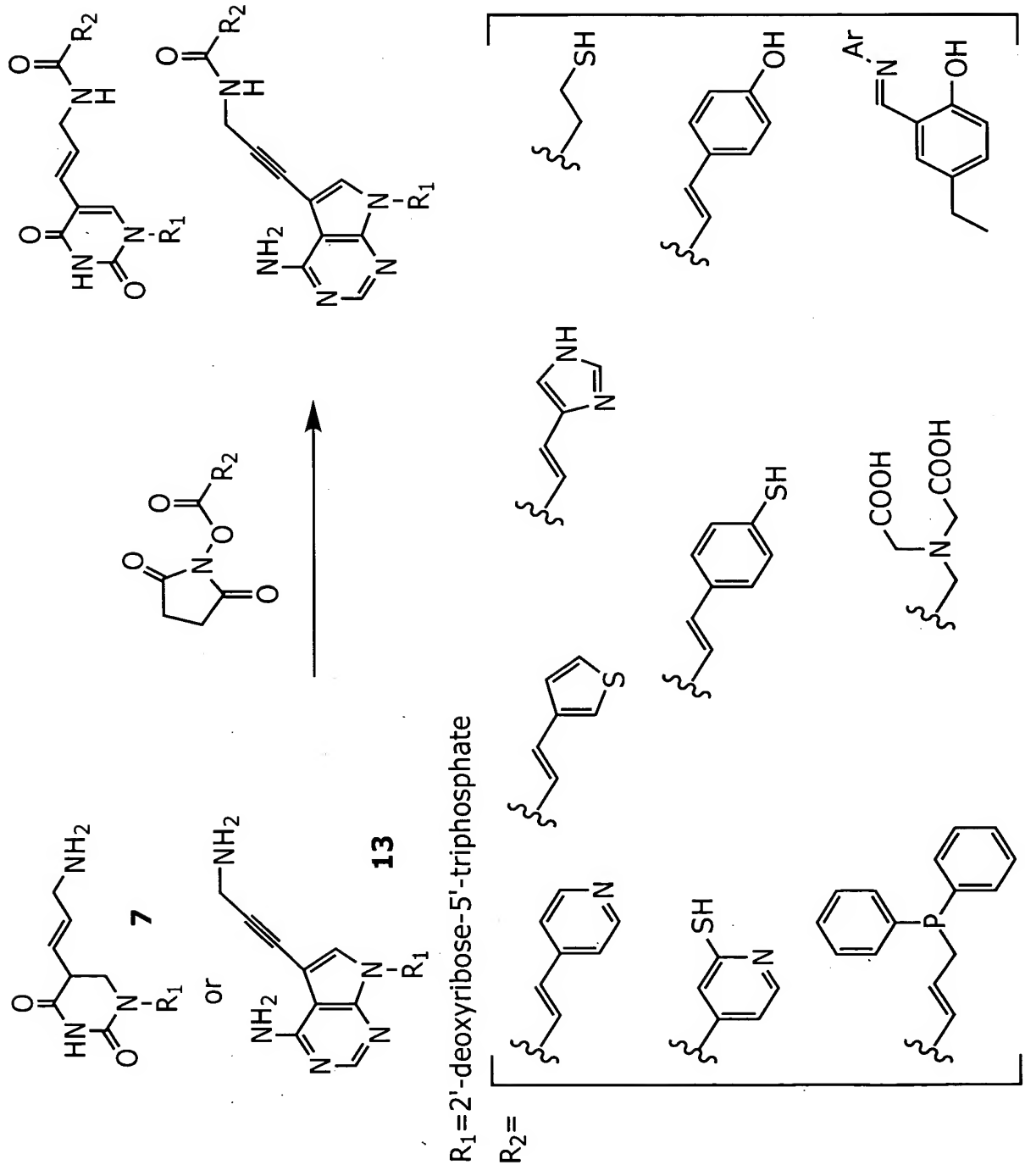

FIG. 68

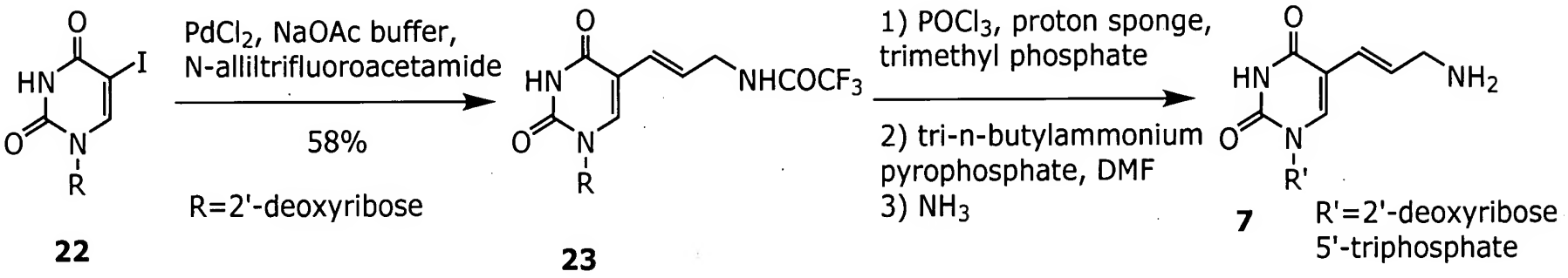

FIG. 69

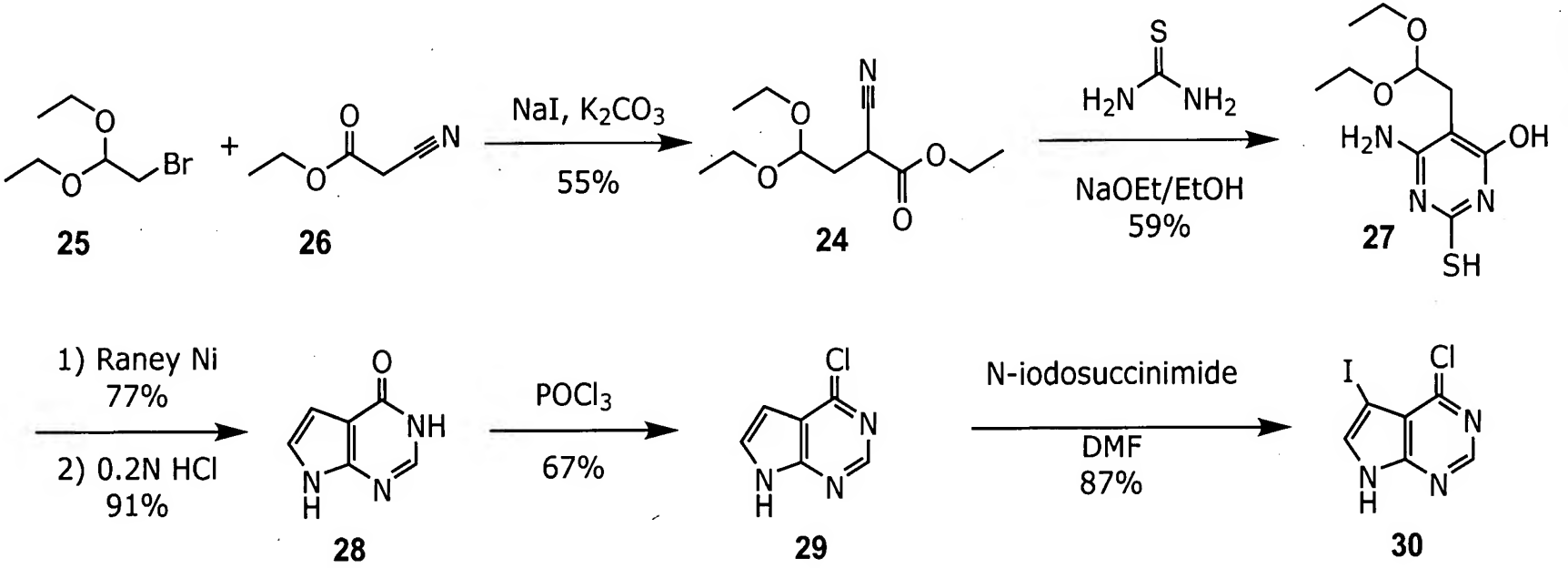

FIG. 70

Title: Evolving New Molecular Function
Inventor: Liu *et al.*
Serial No.: 10/643,752
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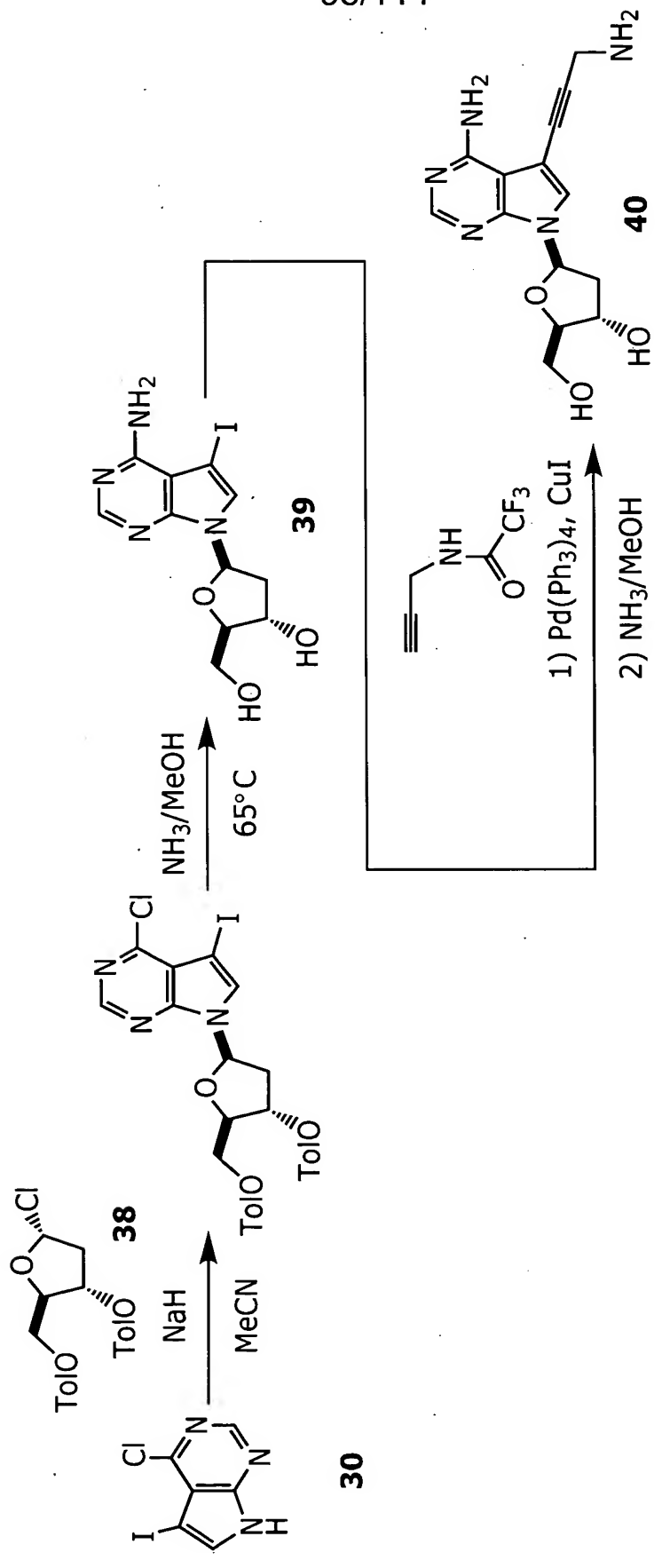


FIG. 71

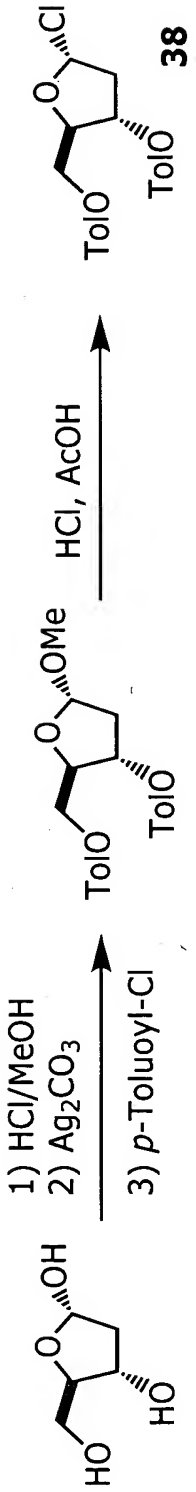
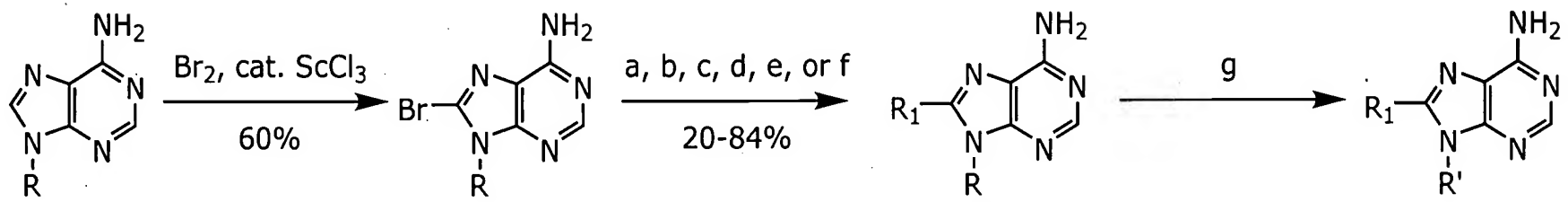


FIG. 72

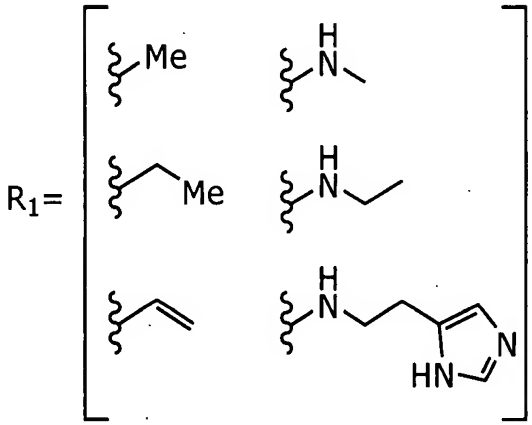
R' = 2'-deoxyribose-5'-triphosphate

FIG. 73



R=2'-deoxyribose

R'=2'-deoxyribose-5'-triphosphate



a (R=Me): 1) HMDS, dioxane, 2) Me_4Sn, $Pd(PPh_3)_4$, NMP, 3) K_2CO_3, MeOH
b (R=Et): 1) HMDS, dioxane, 2) Et_4Sn, $Pd(PPh_3)_4$, NMP, 3) K_2CO_3, MeOH
c (R=CH_2=CH_2): 1) HMDS, dioxane, 2) $(CH_2=CH)_4Sn$, $Pd(PPh_3)_4$, NMP, 3) K_2CO_3, MeOH
d (R=NHMe): $MeNH_2$, H_2O
e (R=NHEt): $EtNH_2$, H_2O
f (R=histaminyl): histamine, EtOH, heat

FIG. 74

Title: Evolving New Molecular Function
Inventor: Liu *et al.*
Serial No.: 10/643,752
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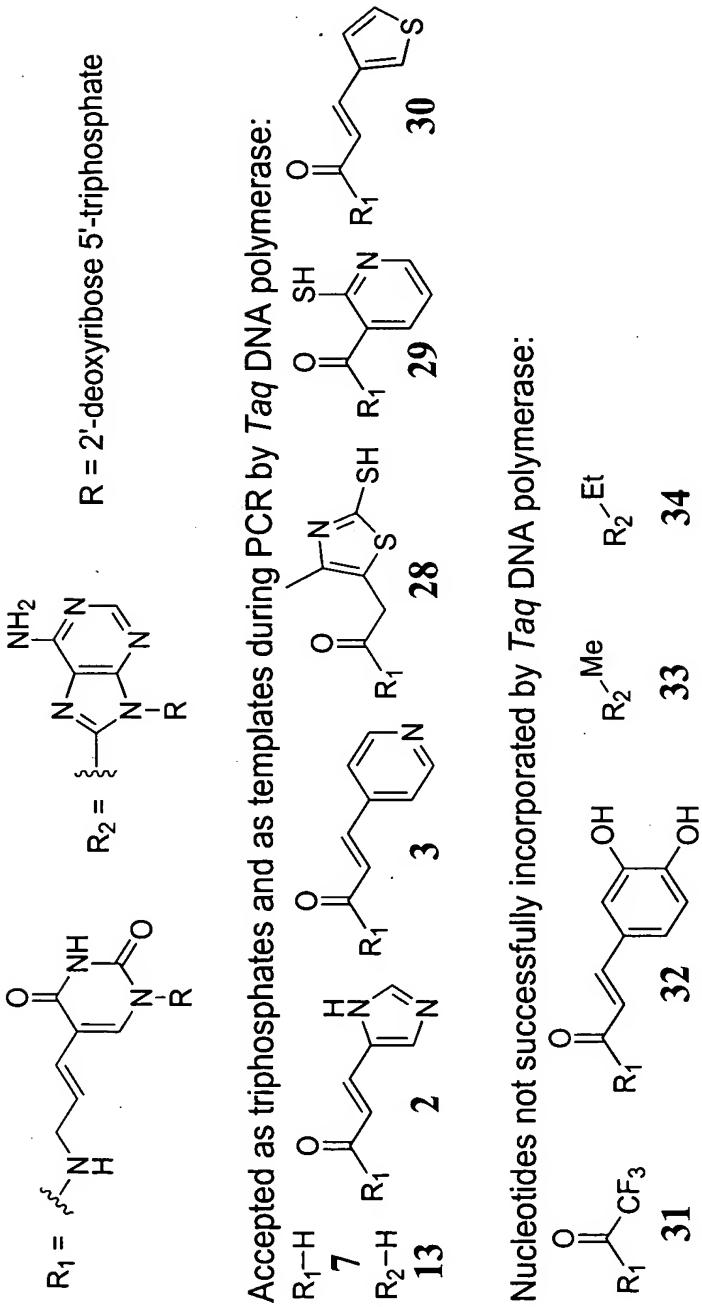
R = 2'-deoxyribose 5'-triphosphate
R1 =
R2 =
Accepted as triphosphates and as templates during PCR by Taq DNA polymerase:
R1–H
7
R2–H
13
2
3
28
29
30
Nucleotides not successfully incorporated by Taq DNA polymerase:
31
32
33
34

FIG. 75

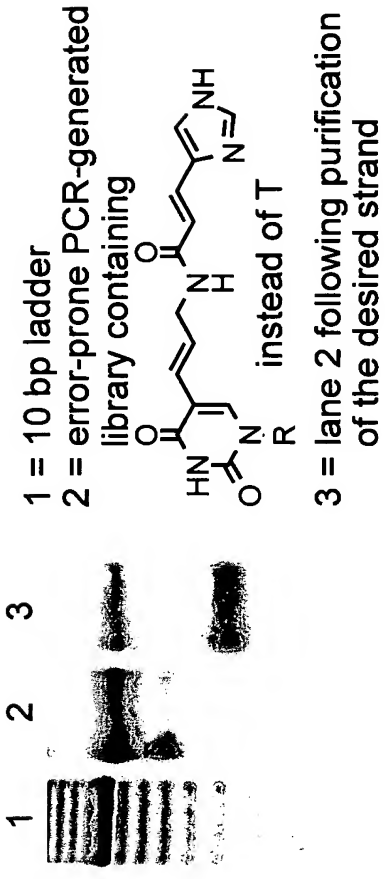
1 = 10 bp ladder
2 = error-prone PCR-generated library containing
instead of T
3 = lane 2 following purification of the desired strand
1
2
3

FIG. 76

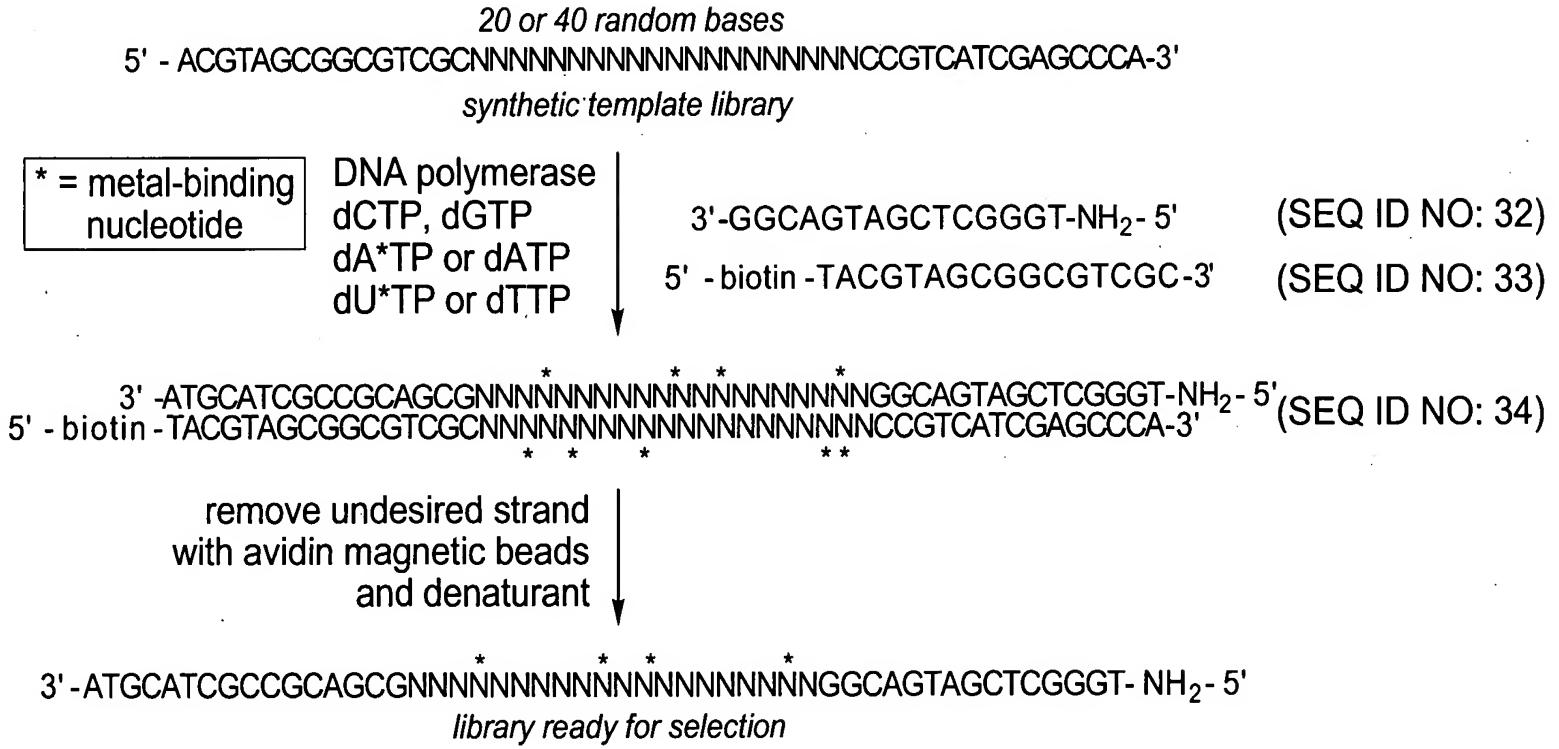

FIG. 77

Title: Evolving New Molecular Function
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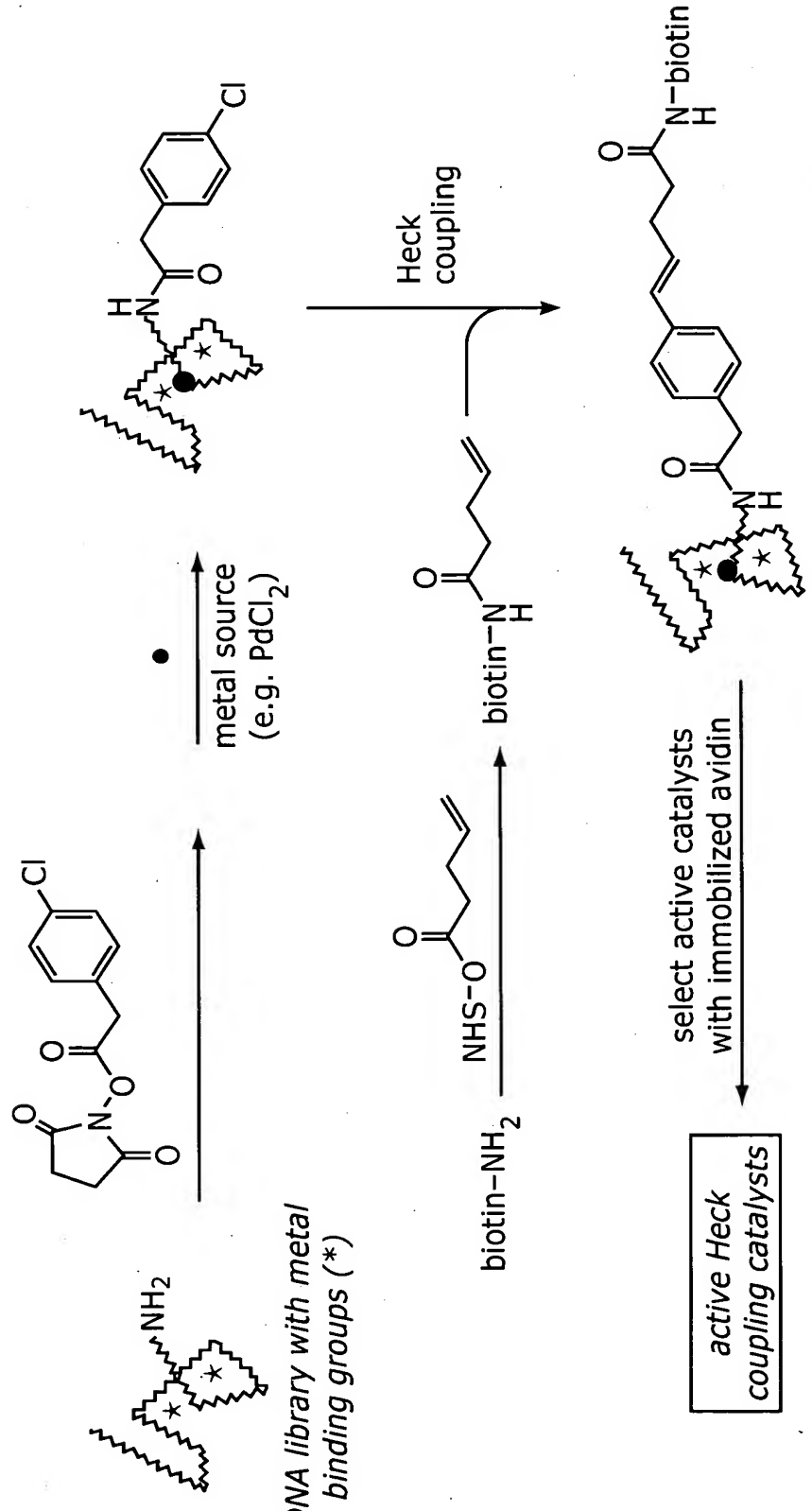

DNA library with metal binding groups (*)
NH2
metal source (e.g. PdCl2)
biotin-NH2
NHS-O
biotin-N
Heck coupling
N-biotin
select active catalysts with immobilized avidin
active Heck coupling catalysts

FIG. 78A

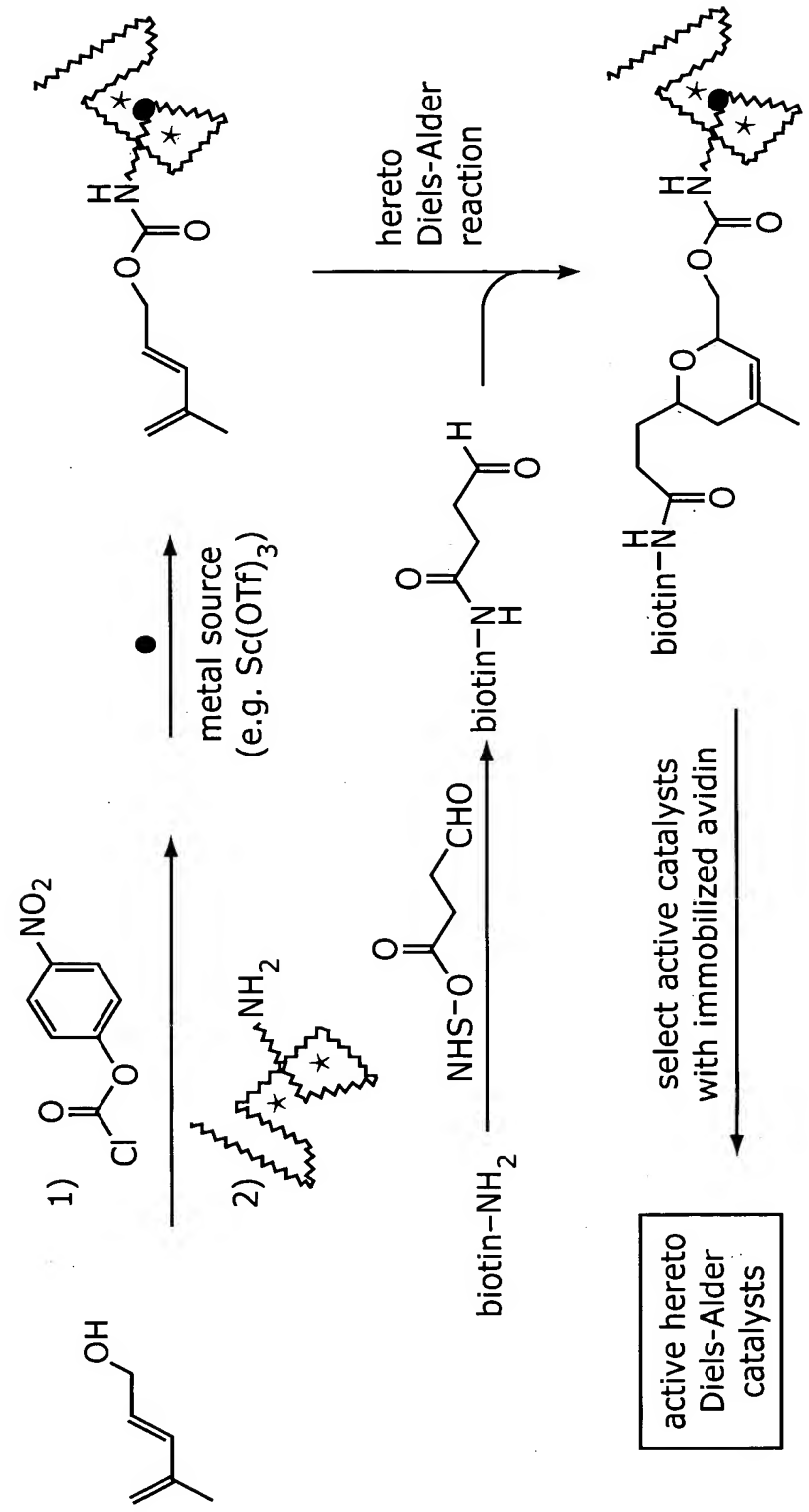

hereto Diels-Alder reaction
metal source (e.g. Sc(OTf)3)
1)
2)
NO2
Cl
O
NH2
CHO
NHS-O
biotin-NH2
biotin-N
H
N
O
OH
select active catalysts with immobilized avidin
active hereto Diels-Alder catalysts

FIG. 78B

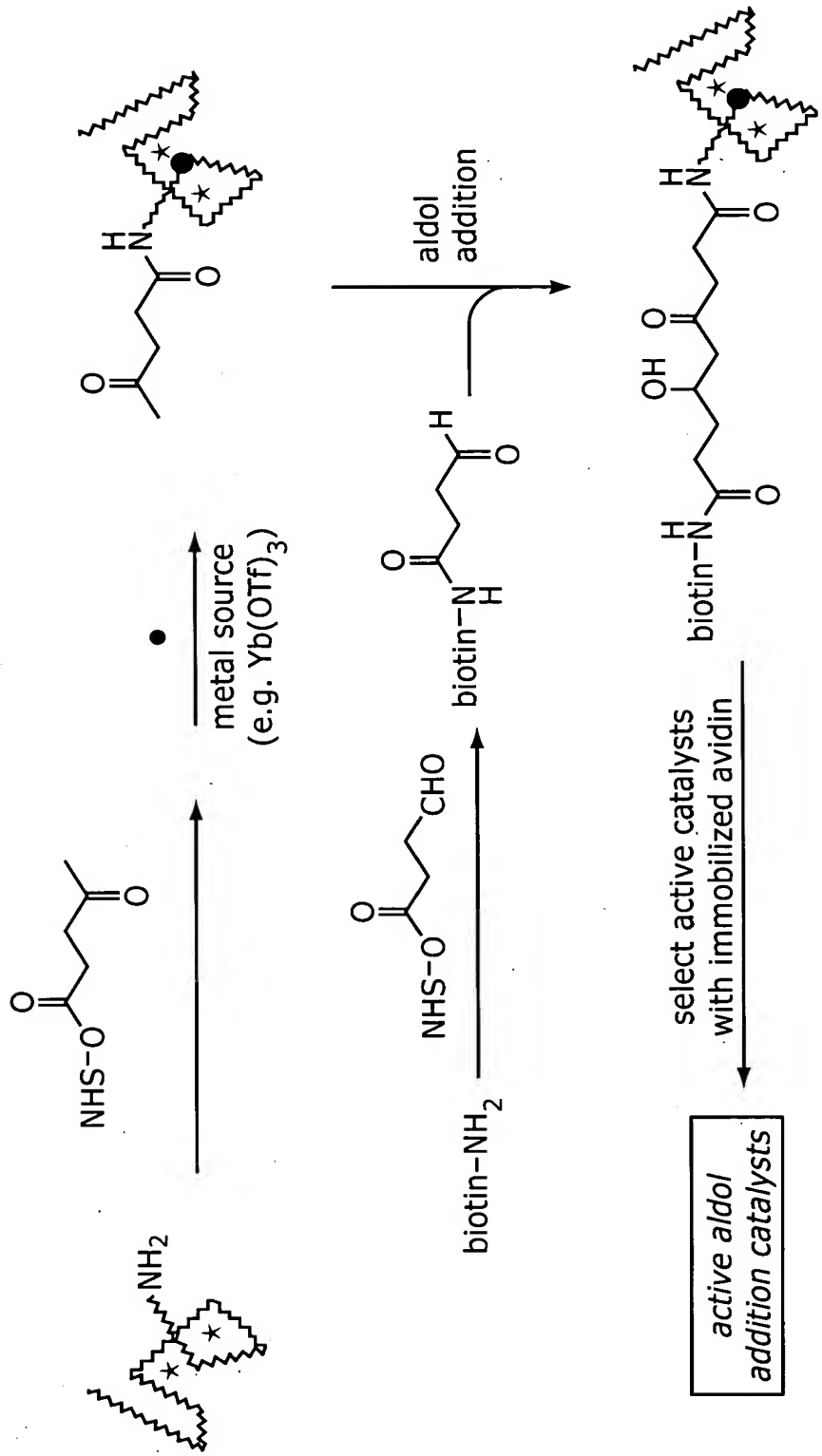
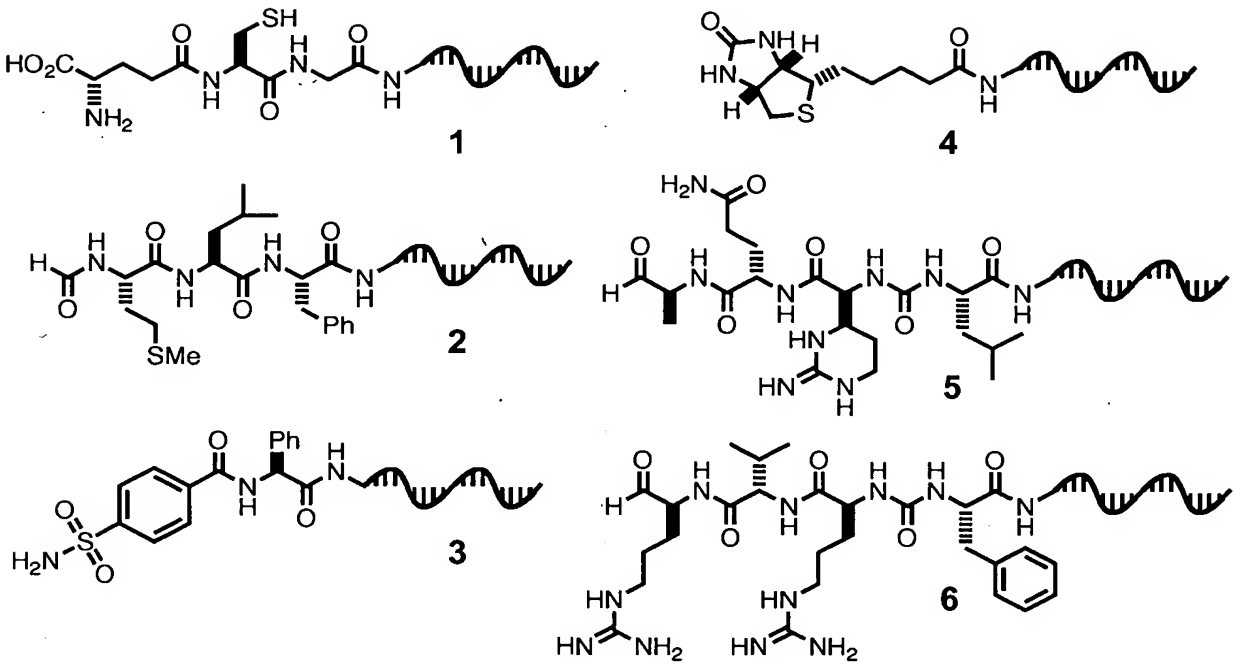

NH2
NHS-O
metal source
(e.g. Yb(OTf)3)
biotin-NH2
CHO
NHS-O
biotin-N
aldol
addition
biotin-N
OH
select active catalysts
with immobilized avidin
active aldol
addition catalysts

FIG. 78C



DNA-linked molecule	target protein	predicted activity	enrichment factor	sensitivity (mol)
1	glutathione *S*-transferase	K_d = 10 μM	2,500	10^{-20}
3	carbonic anhydrase	K_d = 0.9 nM	330	10^{-20}
4	streptavidin	K_d = 40 fM	4,400	10^{-18}
5	papain	IC_{50} = 14 μM	64	10^{-16}
5	chymotrypsin	IC_{50} = 290 nM	76	10^{-16}
6	papain	IC_{50} = 270 nM	98	10^{-18}
6	trypsin	K_d = 100 nM	125	10^{-17}

FIG. 79

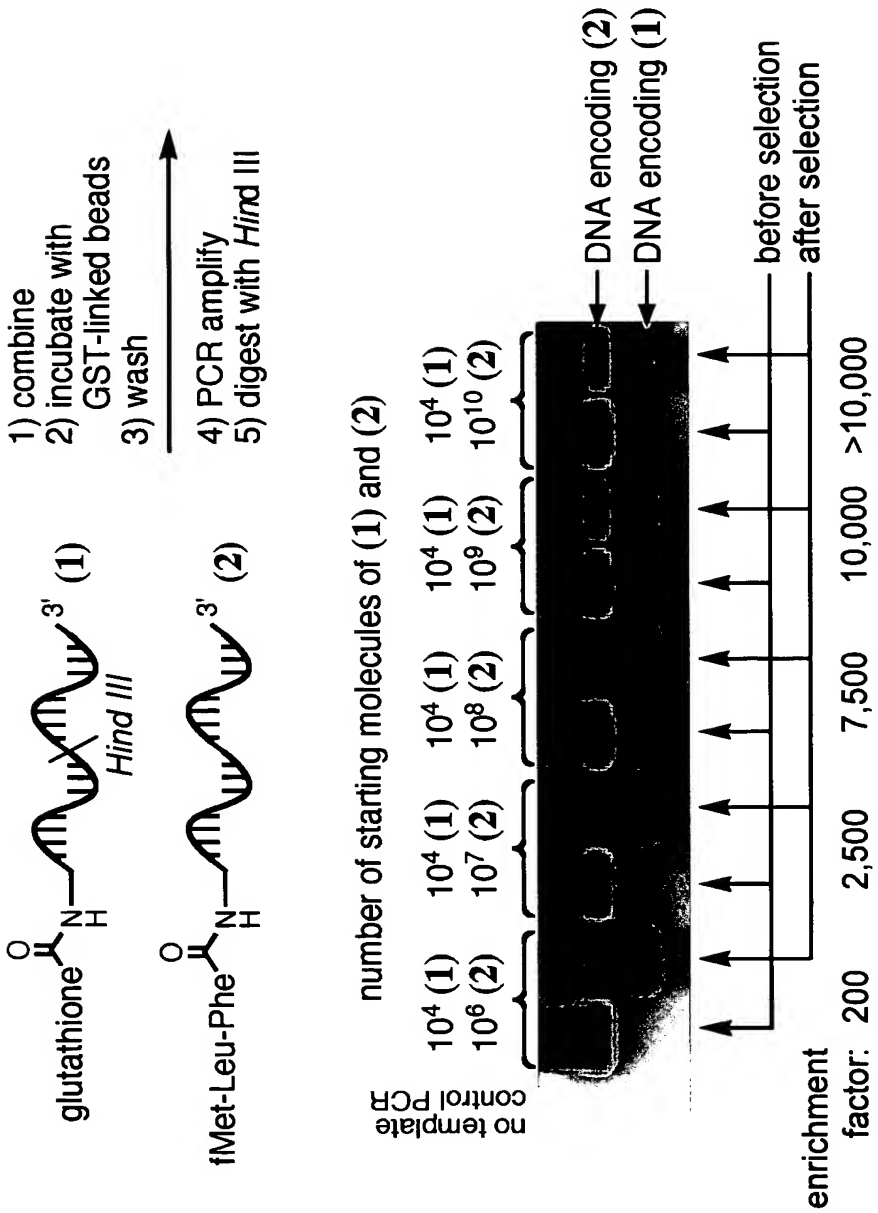

glutathione
fMet-Leu-Phe
Hind III
3'
(1)
(2)
1) combine
2) incubate with GST-linked beads
3) wash
4) PCR amplify
5) digest with Hind III
number of starting molecules of (1) and (2)
no template control PCR
10^4 (1) 10^6 (2)
10^4 (1) 10^7 (2)
10^4 (1) 10^8 (2)
10^4 (1) 10^9 (2)
10^4 (1) 10^10 (2)
DNA encoding (2)
DNA encoding (1)
before selection
after selection
enrichment factor: 200 2,500 7,500 10,000 >10,000

FIG. 80

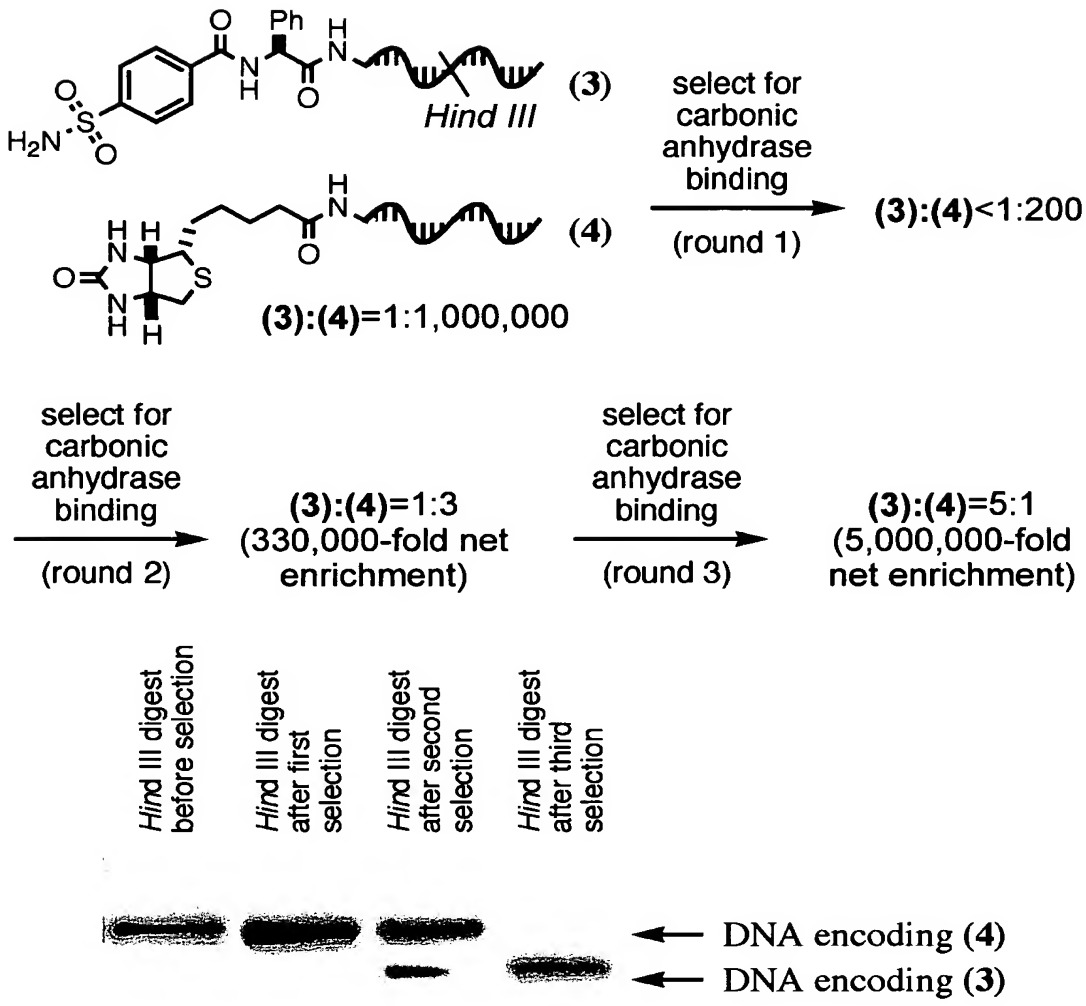
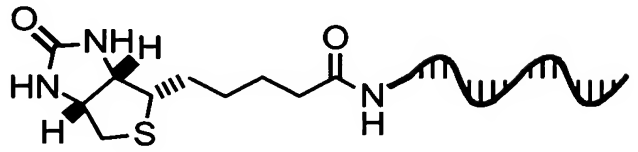
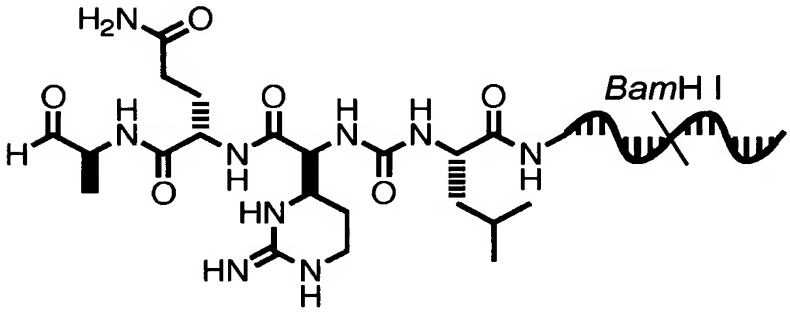

Ph
(3)
Hind III
select for carbonic anhydrase binding
(round 1)
(3):(4)<1:200
(4)
(3):(4)=1:1,000,000
select for carbonic anhydrase binding
(round 2)
(3):(4)=1:3 (330,000-fold net enrichment)
select for carbonic anhydrase binding
(round 3)
(3):(4)=5:1 (5,000,000-fold net enrichment)
Hind III digest before selection
Hind III digest after first selection
Hind III digest after second selection
Hind III digest after third selection
DNA encoding (4)
DNA encoding (3)

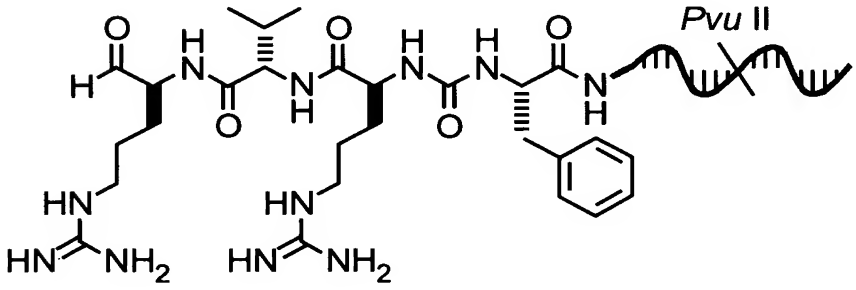
FIG. 81



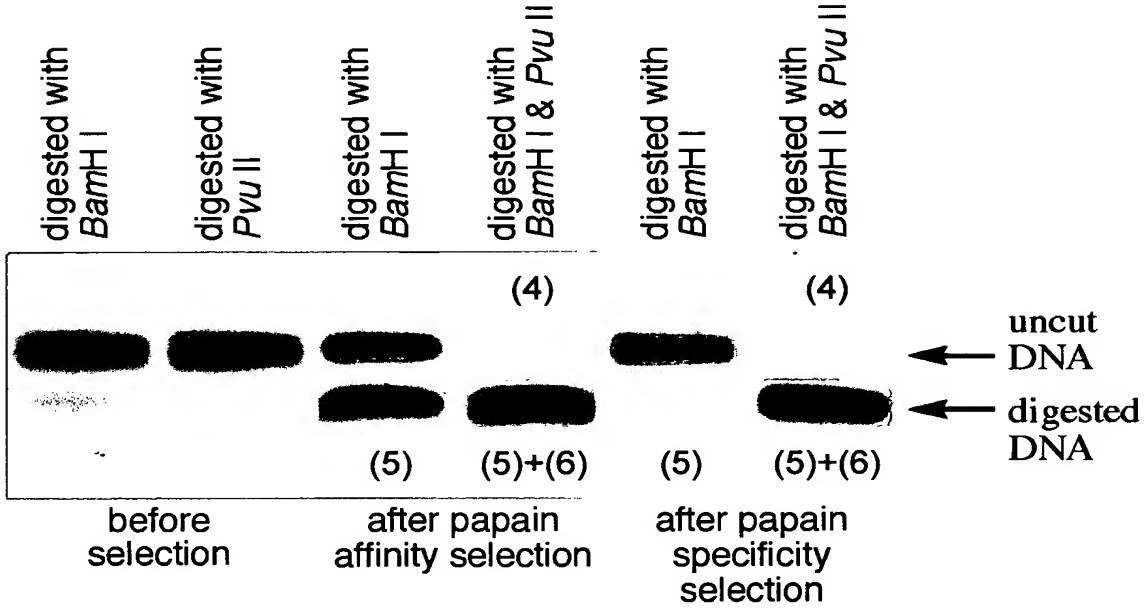
(4)



(5)



(6)



	IC_{50} for chymotrypsin[10c]	IC_{50} for papain[10c]	initial ratio	ratio after papain affinity selection	ratio after papain specificity selection
(4)	>500 μM	>500 μM	24	1	1
(5)	0.29 μM	14 μM	4	12	1
(6)	>500 μM	0.27 μM	1	12	>10

FIG. 82

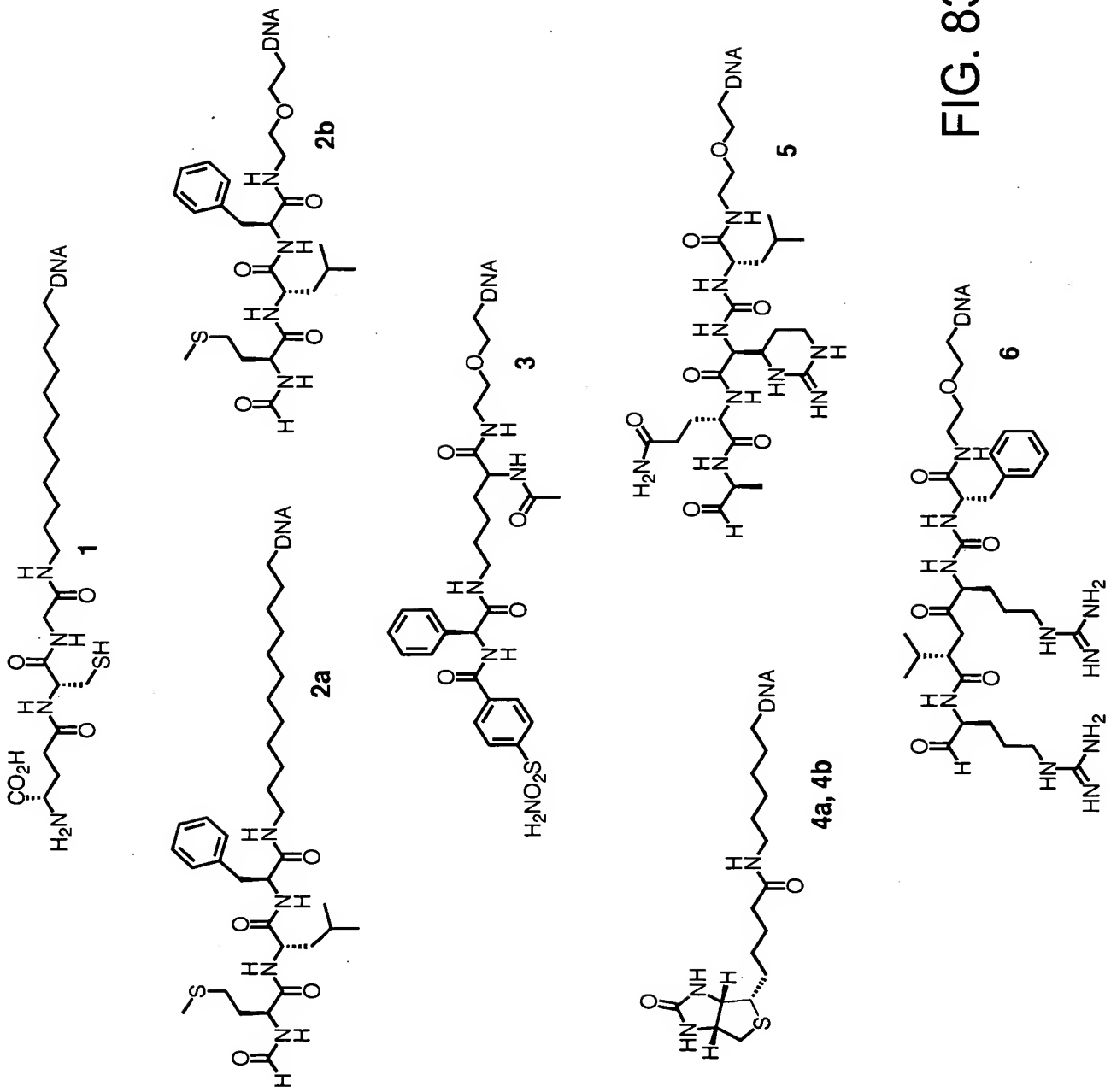


FIG. 83

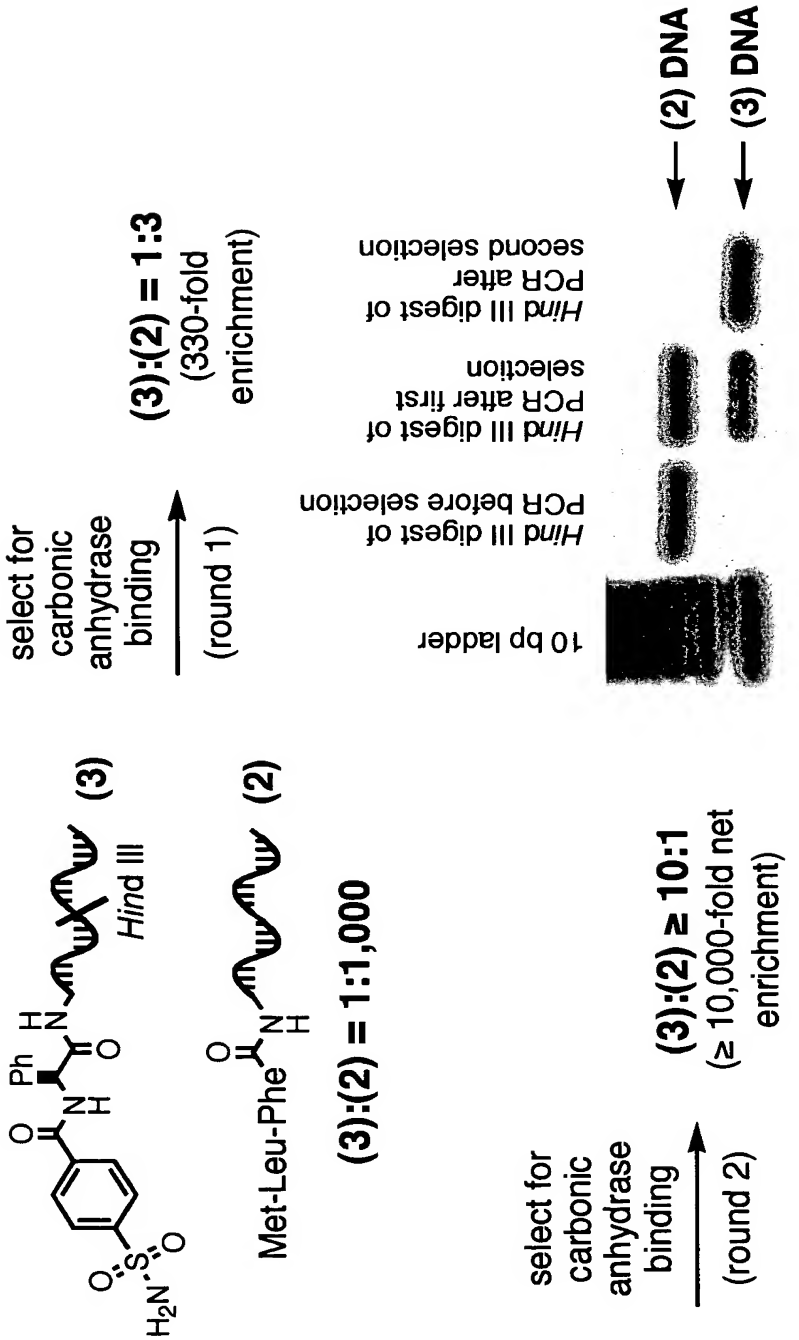

(3)
Hind III
(2)
Met-Leu-Phe
(3):(2) = 1:1,000
select for carbonic anhydrase binding
(round 1)
(3):(2) = 1:3
(330-fold enrichment)
10 bp ladder
Hind III digest of PCR before selection
Hind III digest of PCR after first selection
Hind III digest of PCR after second selection
(2) DNA
(3) DNA
select for carbonic anhydrase binding
(round 2)
(3):(2) ≥ 10:1
(≥ 10,000-fold net enrichment)

FIG. 84

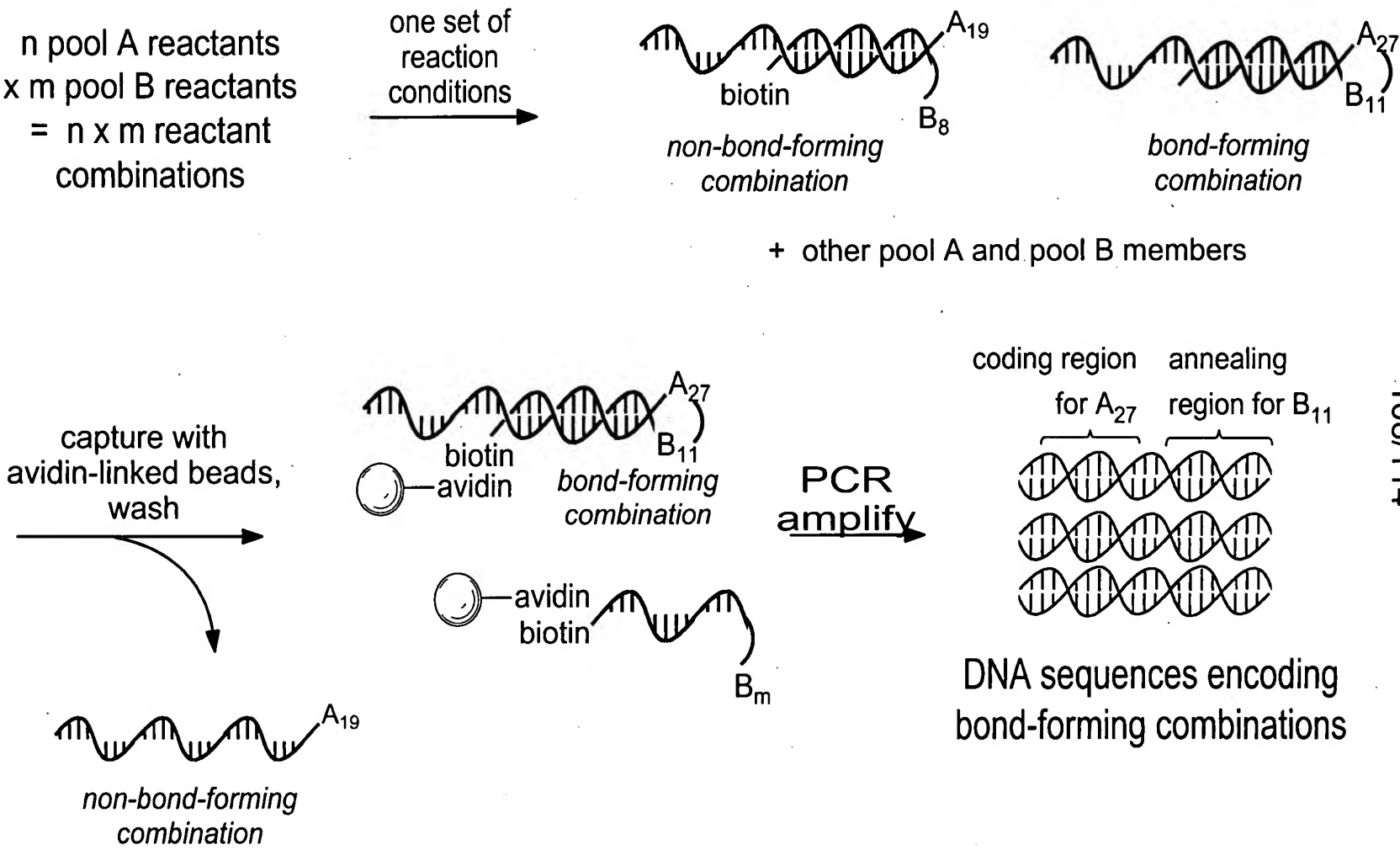


FIG. 85

Title: Evolving New Molecular Function
Inventor: Liu *et al.*
Serial No.: 10/643,752
Atty Docket No.: LSS-001 (9792/5)
Atty/Agent: Brian A. Fairchild
Express Mail Label No.: EL932952135US
Sheet 108 of 114

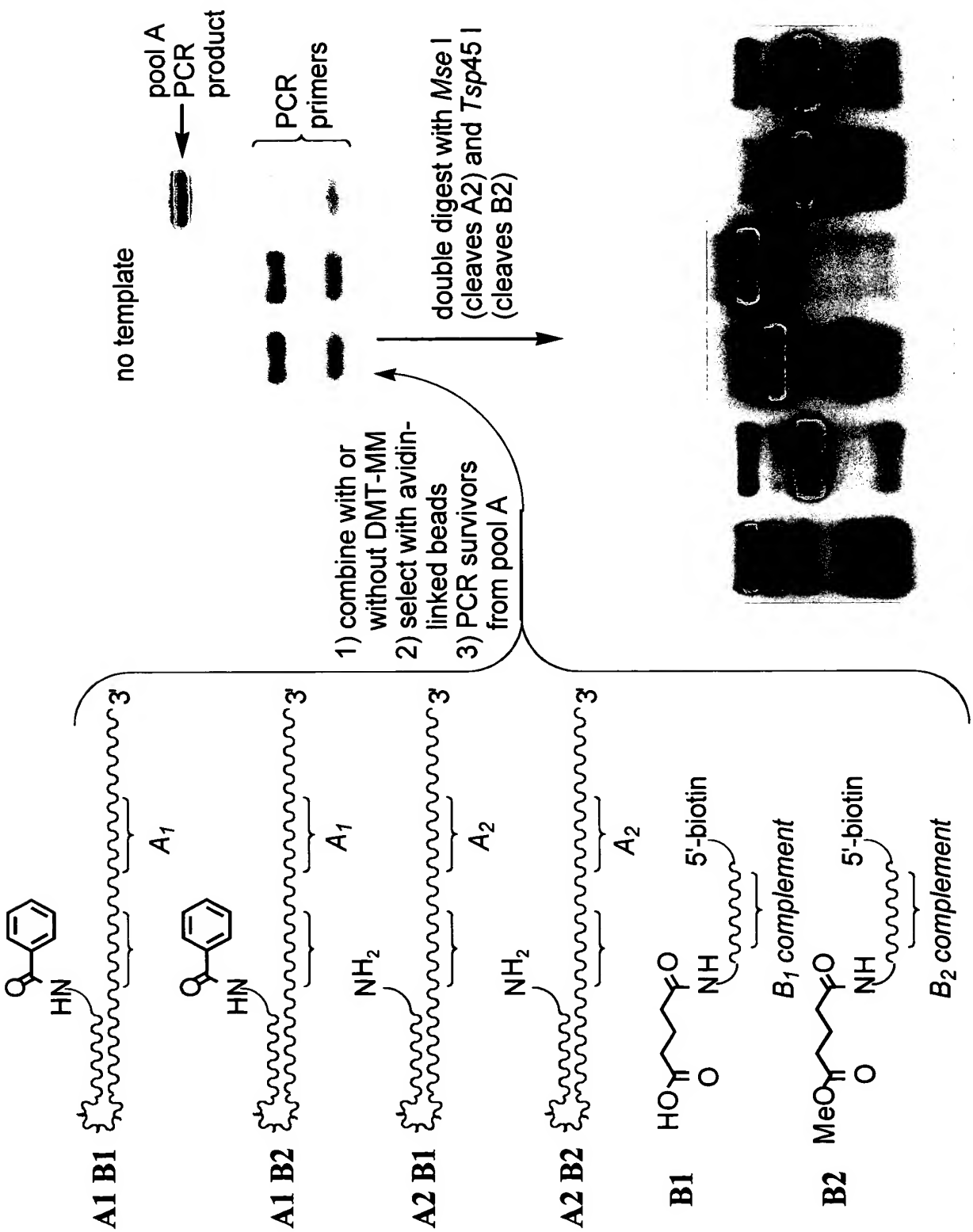
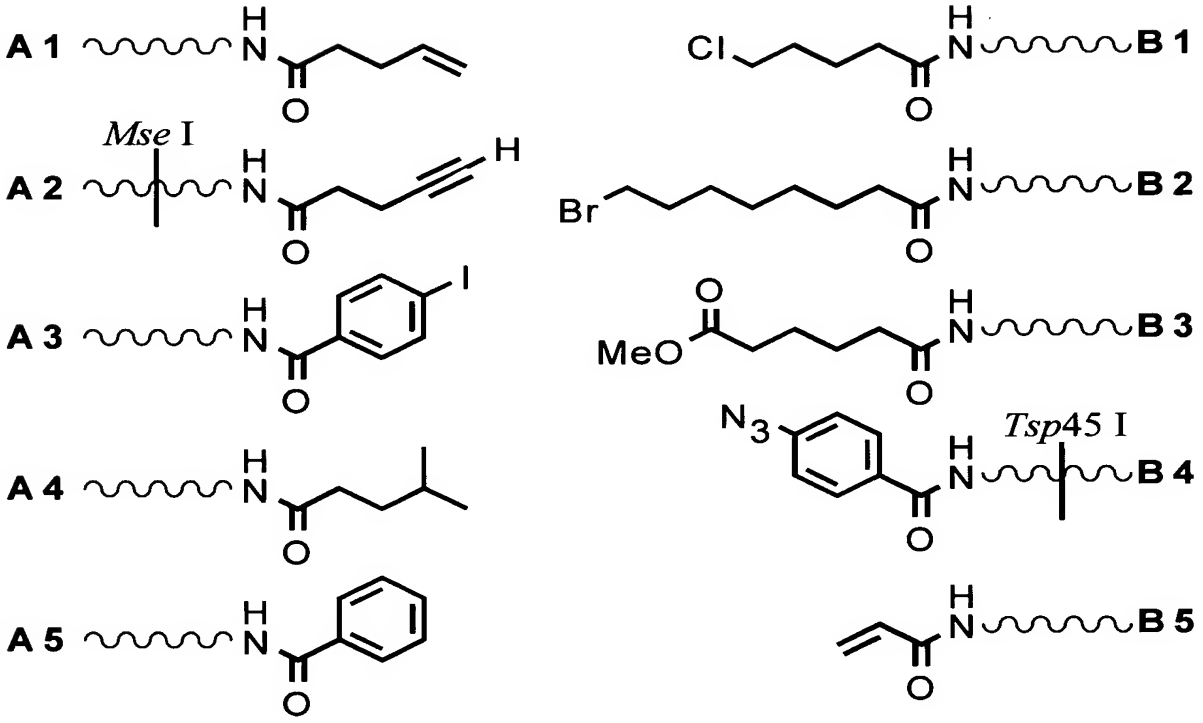

A1 B1
A1 B2
A2 B1
A2 B2
B1
B2
HN
NH2
NH
HO
MeO
O
3'
A1
A2
5'-biotin
B1 complement
B2 complement
1) combine with or without DMT-MM
2) select with avidin-linked beads
3) PCR survivors from pool A
no template
pool A PCR product
PCR primers
double digest with Mse I (cleaves A2) and Tsp45 I (cleaves B2)

FIG. 86



1) combine with or without Cu^+
2) select with avidin-linked beads
3) PCR amplify survivors
4) double digest with *Mse* I (cleaves A2) & *Tsp*45 I (cleaves B4)

FIG. 87

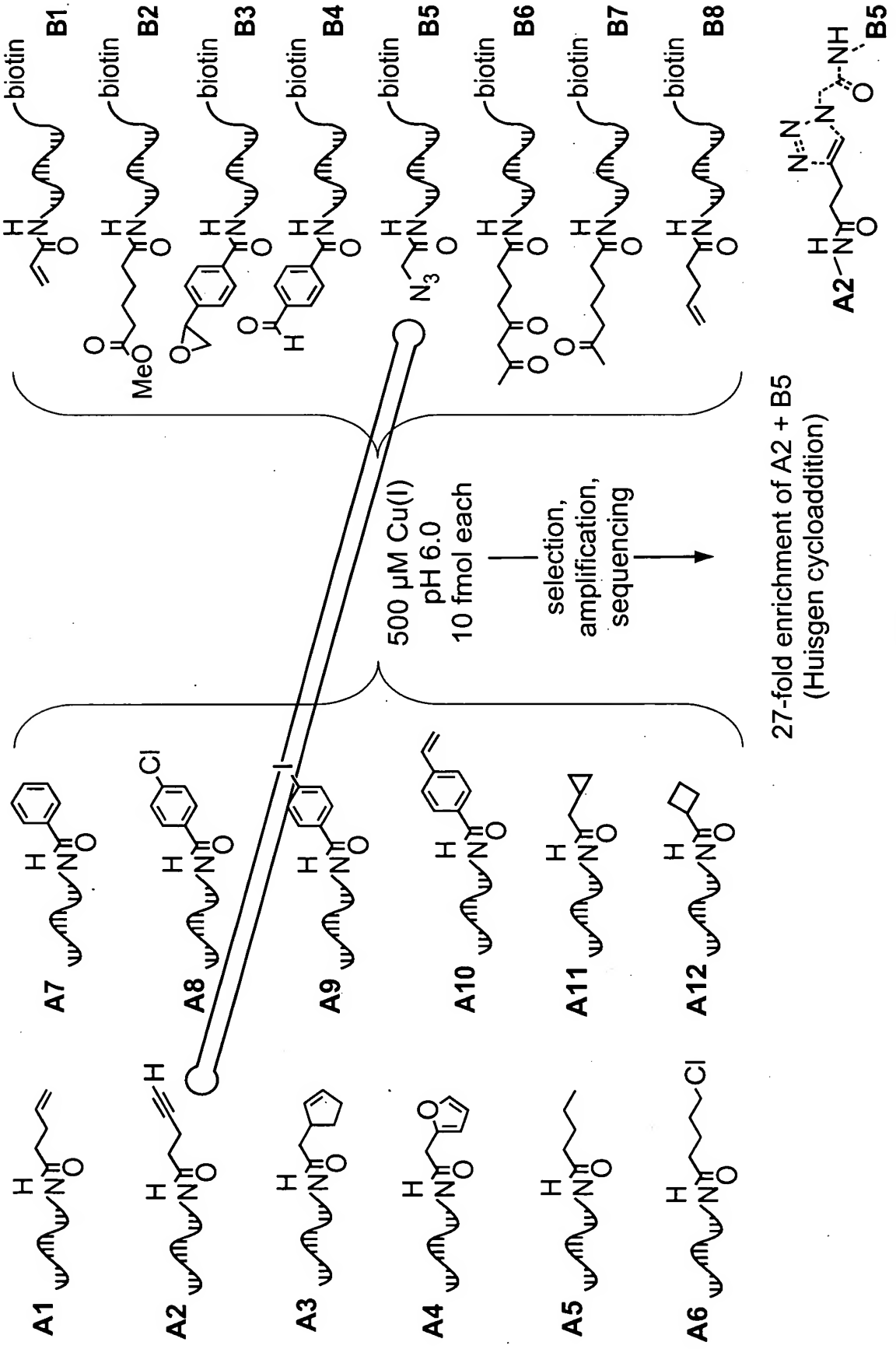

biotin
B1
B2
B3
B4
B5
B6
B7
B8
MeO
N_3
A1
A2
A3
A4
A5
A6
A7
A8
A9
A10
A11
A12
Cl
500 µM Cu(I)
pH 6.0
10 fmol each
selection,
amplification,
sequencing
27-fold enrichment of A2 + B5
(Huisgen cycloaddition)
A2
B5
NH

FIG. 88

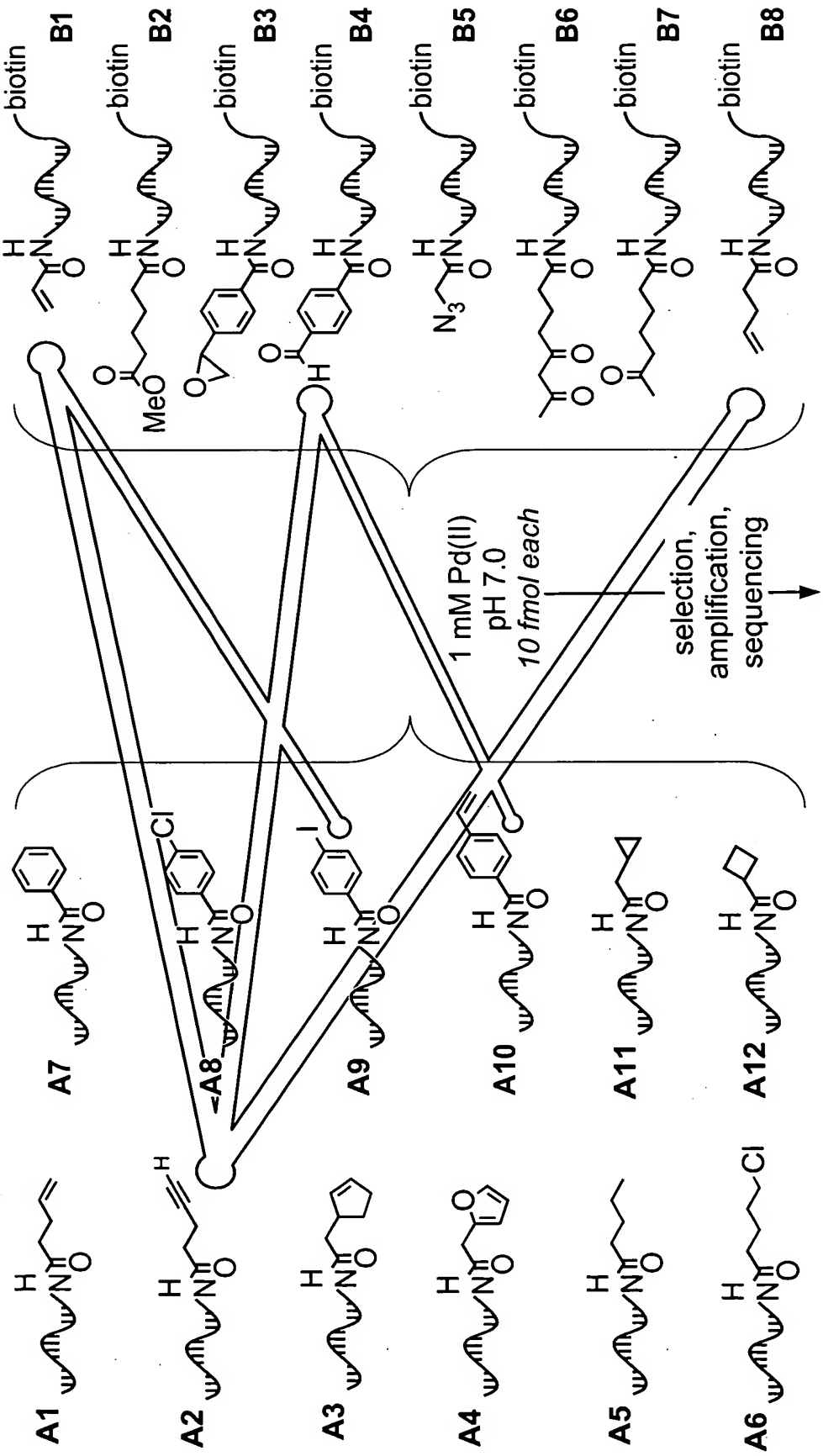

B1
B2
B3
B4
B5
B6
B7
B8
biotin
MeO
N_3
1 mM Pd(II)
pH 7.0
10 fmol each
selection,
amplification,
sequencing
A1
A2
A3
A4
A5
A6
A7
A8
A9
A10
A11
A12
Cl
I

FIG. 89

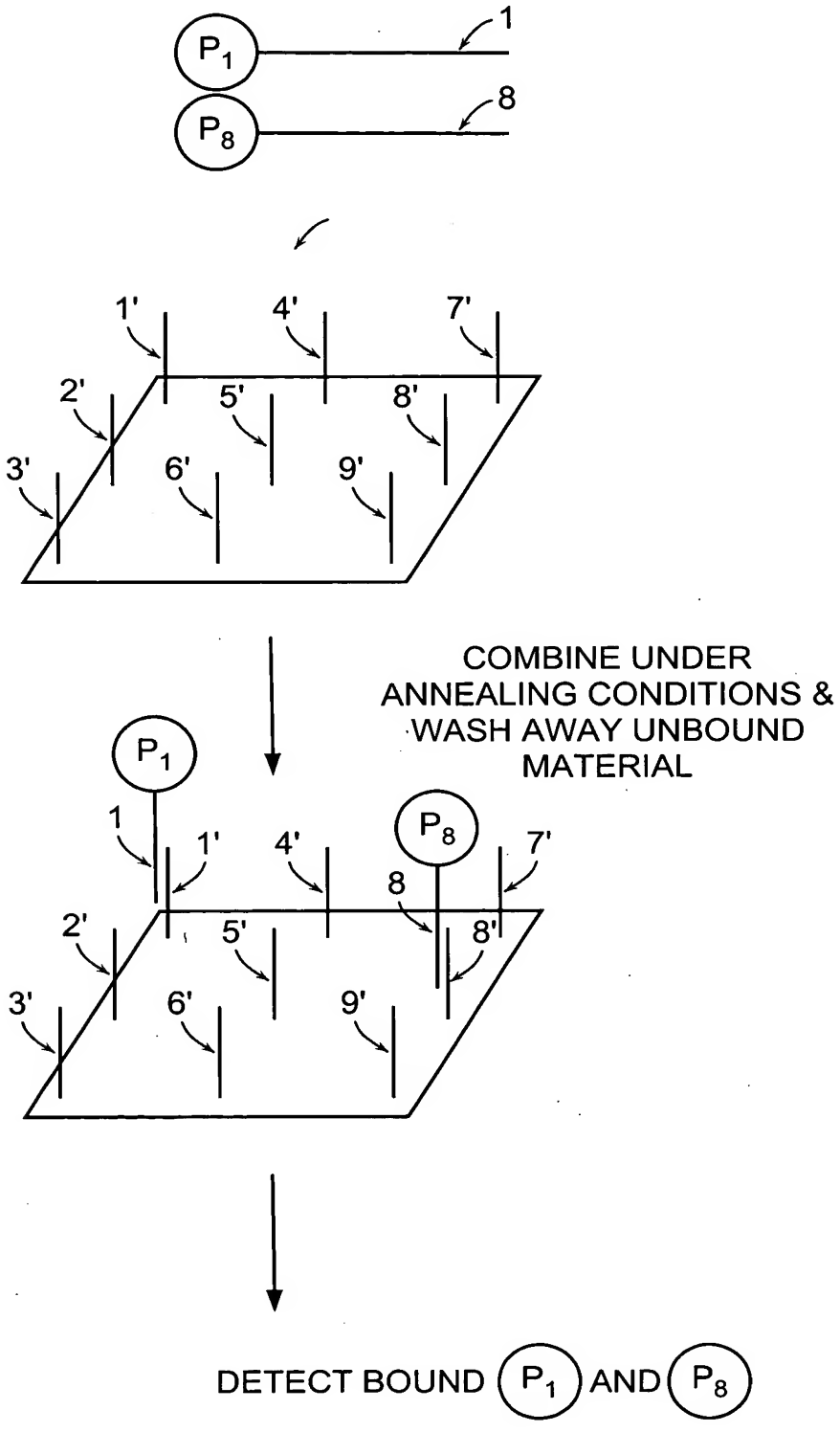

P1
1
P8
8
1'
4'
7'
2'
5'
8'
3'
6'
9'
COMBINE UNDER ANNEALING CONDITIONS & WASH AWAY UNBOUND MATERIAL
P1
1
1'
P8
4'
7'
8
2'
5'
8'
3'
6'
9'
DETECT BOUND P1 AND P8

FIG. 90

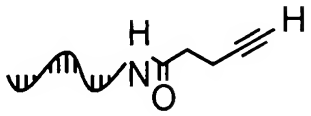
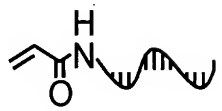
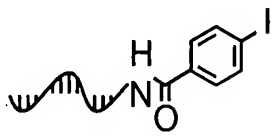
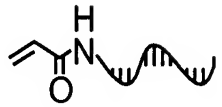
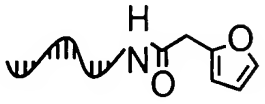
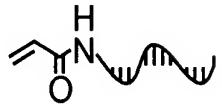
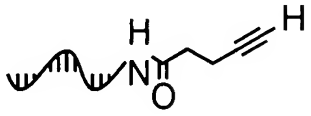
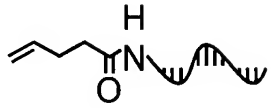
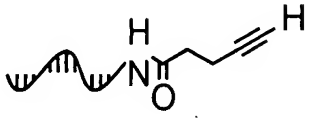
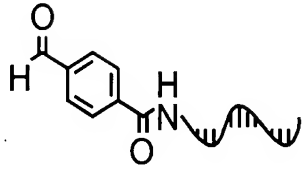
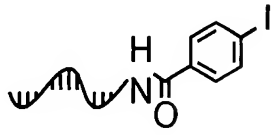
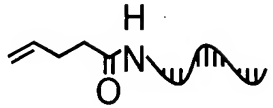
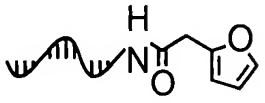
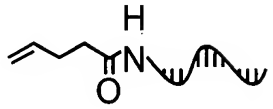
			ARRAY SIGNAL ÷ BACKGROUND	DNA-TEMPLATED REACTION YIELD
	+		78-fold	75-95%
	+	 (Heck)	76-fold	71-91%
	+		56-fold	70-90%
	+		44-fold	75-95%
	+		38-fold	53-73%
	+	 (Heck)	30-fold	57-77%
	+		22-fold	75-95%

FIG. 91